

## Highly fluorinated amphiphiles and colloidal systems, and their applications in the biomedical field. A contribution

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**Summary** — Fluorocarbons and fluorocarbon moieties are uniquely characterized by very strong intramolecular bonds and very weak intermolecular interactions. This results in a combination of exceptional thermal, chemical and biological inertness, low surface tension, high fluidity, excellent spreading characteristics, low solubility in water, and high gas dissolving capacities, which are the basis for innovative applications in the biomedical field. Perfluoroalkyl chains are larger and more rigid than their hydrogenated counterparts. They are considerably more hydrophobic, and are lipophobic as well. A large variety of well-defined, modular fluorinated surfactants whose polar head groups consist of polyols, sugars, sugar phosphates, amino acids, amine oxides, phosphocholine, phosphatidylcholine, etc. has recently been synthesized. Fluorinated surfactants are significantly more surface active than their hydrocarbon counterparts, both in terms of effectiveness and of efficiency. Despite this, they are less hemolytic and less detergent. Fluorosurfactants appear unable to extract membrane proteins. Fluorinated chains confer to surfactants a powerful driving force for collecting and organizing at interfaces. As compared to non-fluorinated analogs, fluorosurfactants have also a much stronger capacity to self-aggregate into discrete molecular assemblies when dispersed in water and other solvents. Even very short, single-chain fluorinated amphiphiles can form highly stable, heat-sterilizable vesicles, without the need for supplementary associative interactions. Sturdy microtubules were obtained from non-chiral, non-hydrogen bonding single-chain fluorosurfactants. Fluorinated amphiphiles can be used to engineer a variety of colloidal systems and manipulate their morphology, structure and properties. Stable fluorinated films, membranes and vesicles can also be prepared from combinations of standard surfactants with fluorocarbon/hydrocarbon diblock molecules. In bilayer membranes made from fluorinated amphiphiles the fluorinated tails segregate to form an internal teflon-like hydrophobic and lipophobic film that increases the stability of the membrane and reduces its permeability. This fluorinated film can also influence the behavior of fluorinated vesicles in a biological milieu. For example, it can affect the *in vivo* recognition and fate of particles, or the enzymatic hydrolysis of phospholipid components. Major applications of fluorocarbons currently in advanced clinical trials include injectable emulsions for delivering oxygen to tissues at risk of hypoxia; a neat fluorocarbon for treatment of acute respiratory failure by liquid ventilation; and gaseous fluorocarbon-stabilized microbubbles for use as contrast agents for ultrasound imaging. Fluorosurfactants also allow the preparation of a range of stable direct and reverse emulsions, microemulsions, multiple emulsions, and gels, some of which may include fluorocarbon and hydrocarbon and aqueous phases simultaneously. Highly fluorinated systems have potential for the delivery of drugs, prodrugs, vaccines, genes, markers, contrast agents and other materials (© Société française de biochimie et biologie moléculaire / Elsevier, Paris).

**fluorocarbons / fluorinated amphiphiles / fluorocarbon emulsions / gels / vesicles / liposomes / tubules / bilayers / Langmuir monolayers / oxygen delivery / drug delivery / diagnosis**

### Introduction

Fluorine is the most electronegative of all elements, and its dense electron cloud has very low polarizability. Replacing the hydrogen atoms by fluorines is certainly one of the most dramatic perturbations which can be inflicted on an organic molecule. Perfluorocarbons (hydrocarbons in which all hydrogens are replaced by fluorines) and perfluorocarbon moieties have very strong intramolecular bonds and very weak intermolecular interactions. It is no wonder that perfluorocarbons (fluorocarbons) have properties that differ significantly from those of the parent hydrocarbons, and in many unique ways. Fluorocarbons substantially surpass hydrocarbons in terms of stability, surface activity and hydrophobicity. They also have higher fluidity, lower dielectric constants, higher compressibility and higher gas-dissolving

capacities. Fluorocarbons are not only extremely hydrophobic, they are lipophobic as well. This provides a potent driving force for fluorocarbon moieties to segregate from both lipidic and hydrophilic moieties, and for fluorinated amphiphiles to self-assemble into highly stable and well organized films, bilayers, and discrete supramolecular system such as vesicles, tubules, etc. Fluorinated amphiphiles provide versatile tools for adjusting the properties of such organized disperse systems. Fluorocarbons and fluorinated surfactants (fluorosurfactants) also allow the formulation of a wealth of multi-component, multi-phase compartmentalized colloidal systems.

This short, non-exhaustive review intends to illustrate the role that highly fluorinated components may play in colloid and surface chemistry. It consists primarily of an account of the authors' own efforts and experience in the

field, hence will be limited to some specific aspects of this chemistry. It also intends to outline the potential applications of highly fluorinated compounds and systems in biomedical research and in the development of novel therapies.

## Fluorocarbons and fluorosurfactants

### Specific characteristics of fluorocarbons

Carbon-fluorine bonds are, on average, more stable than carbon-hydrogen bonds by approximately 75 kJ mol<sup>-1</sup> [1]. Perfluorination also reinforces the skeleton's C-C bonds as a consequence of strong inductive effects. The stronger intramolecular bonds in fluorocarbons, as compared to hydrocarbons, are largely responsible for the exceptional thermal and chemical stability of fluorocarbons. Fluorocarbons resist to extremely corrosive environments. No bacteria are known to feed on fluorocarbons, and no enzymatic degradation of such compounds has been reported.

Fluorine's electronegativity, its larger size as compared to hydrogen, and its dense electron cloud result in a compact, repellent sheath of electrons that protects the perfluorinated moiety and its surroundings against the approach of other molecular species, including reagents. This sort of 'Scotchguard®' effect, at the molecular level, certainly contributes to rendering perfluoroalkyl chains inert.

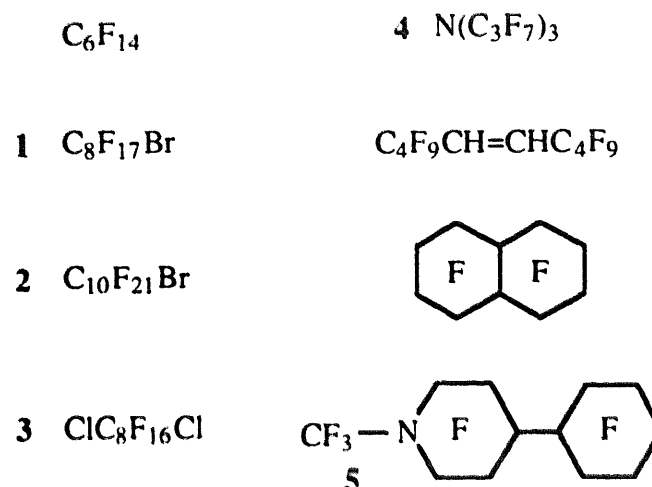
Perfluoroalkyl (*F*-alkyl) chains, *ie* chains in which all the hydrogen atoms have been replaced by fluorine atoms, are bulkier and more rigid than their hydrogenated counterparts, with cross-sections of *ca* 30 Å<sup>2</sup> vs 20 Å<sup>2</sup>. The *F*-alkyl chain's conformational freedom is strongly reduced, as well as the occurrence of gauche defects [2].

The low polarizability of the fluorine atom results in low van der Waals forces between molecules and low cohesive energy densities in liquid fluorocarbons [3]. This is responsible for many of the most valuable and unmatched properties of fluorocarbons, including their low surface tension, high fluidity and spreading characteristics, extreme hydrophobicity and simultaneous lipophobicity, high vapor pressure, high compressibility and exceptional gas-dissolving capacities. Additional properties relevant to fluorocarbons' potential in the biomedical field include high density, non-adherence and antifriction properties, and magnetic susceptibility values close to those of water.

The perfluorochemicals that are being evaluated for *in vivo* oxygen delivery, liquid ventilation, diagnosis and drug delivery can be linear or cyclic, and may contain some hydrogen, halogen, oxygen, or nitrogen atoms. The compounds which have been most thoroughly investigated for biomedical applications are shown in figure 1 [4].

### Fluorosurfactants

Introducing a *F*-alkyl chain into a molecule usually renders this molecule strongly amphiphilic or further enhances an



**Fig 1.** Fluorocarbons most thoroughly investigated for use in therapeutic applications [4, 44].

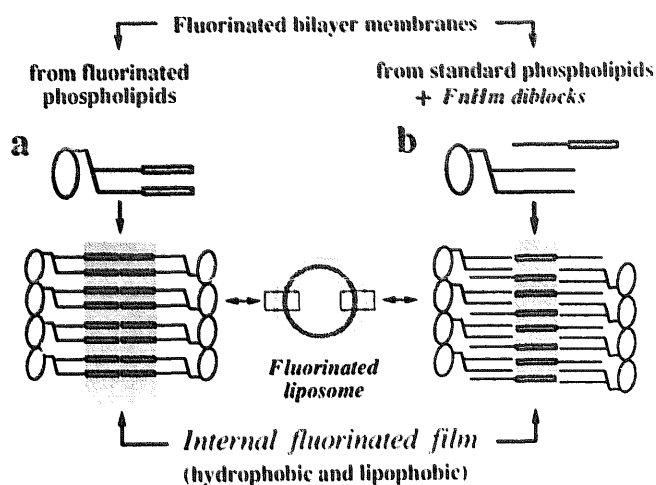
existing amphiphilic character [5, 6]. Fluorosurfactants (surfactants with one or more *F*-alkyl hydrophobic chains) are considerably more surface active than their hydrogenated analogs. Surface tensions in the 15 to 20 mN m<sup>-1</sup> range can easily be attained.

Fluorosurfactants are of course best suited for effectively reducing fluorocarbon/water interfacial tensions, as for preparing fluorocarbon emulsions and microemulsions; interfacial tension values on the order of 1 mN m<sup>-1</sup> are readily obtained. Fluorosurfactants are also very efficient, as their critical micellar concentration (cmc) is commonly two orders of magnitude lower than for hydrocarbon surfactants of similar length. Fluorosurfactants present enhanced resistance to biological fluids or corrosive environments.

The mixing of fluorocarbons and hydrocarbons is highly non-ideal [7], resulting largely from the difference in cohesive energy between the two types of molecules. Likewise, fluorinated amphiphiles and hydrogenated amphiphiles show only limited miscibility. When present simultaneously in micelles [8], monolayers, or liposomes, the two types of amphiphiles tend to form phase-separated domains [9]. Such phase separated liposomes were used to simulate the hole formation process that occurs when a macrophage attacks a tumor cell [9].

In self-assemblies of amphiphiles which have both fluorocarbon and hydrocarbon segments in their structure, fluorinated chains tend to regroup and segregate from both the hydrophilic and the lipidic moieties, each clustering in a separate zone. The case of bilayer membranes is illustrated in figure 2. This segregation phenomenon is also expected to reduce the jutting that normally exists between fatty acid chains in bilayers made from phospholipids.

As noted, fluorocarbons and fluorocarbon moieties are significantly more hydrophobic than their hydrogenated analogs. The solubility of CF<sub>4</sub> in water, for example, is one



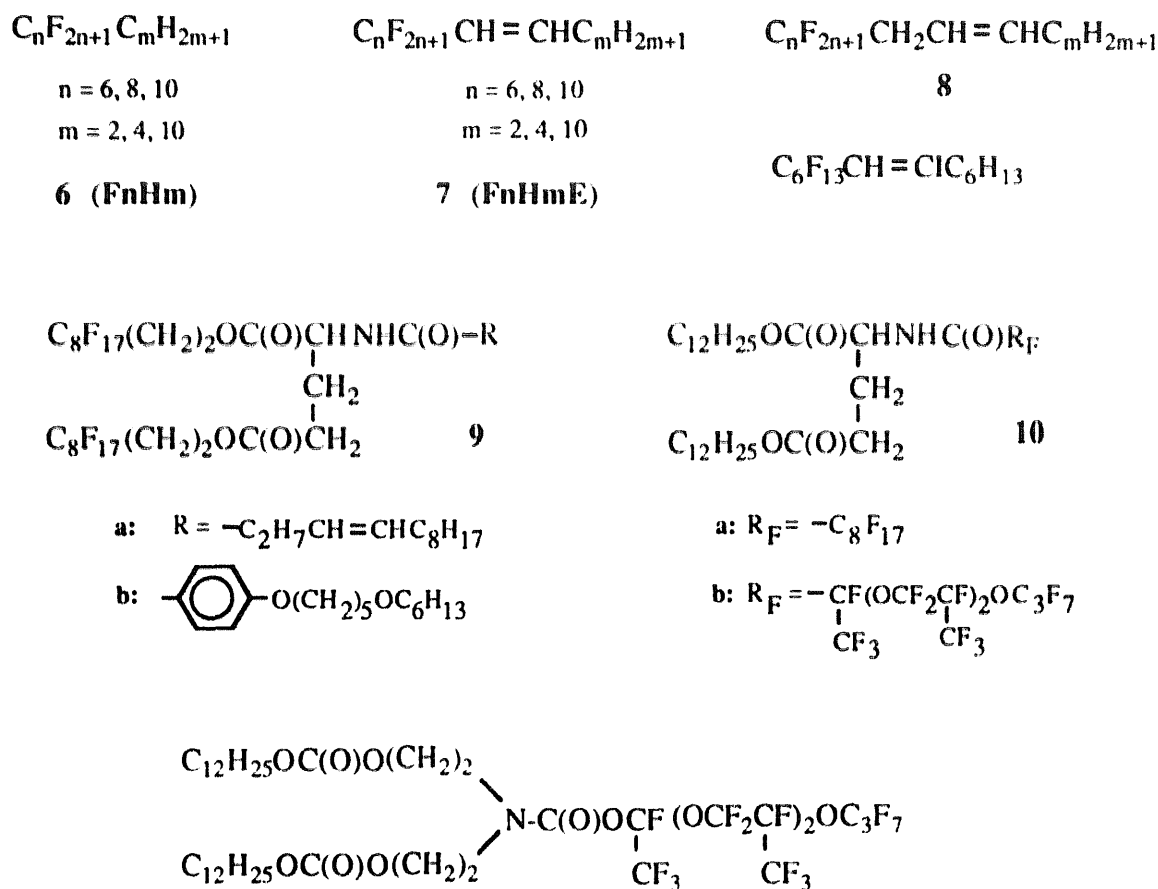
**Fig 2.** Fluorinated bilayer membranes and fluorinated liposomes can be obtained from fluorinated phospholipids (**a**) and from a combination of standard phospholipids and fluorocarbon-hydrocarbon diblocks (**b**). In both cases, the clustering of the fluorinated chains (grey bars) results in the formation of a hydrophobic and lipophobic internal fluorinated, teflon-like film within the bilayer membrane. From [26].

order of magnitude lower than that of  $\text{CH}_4$  [10]. The hydrophobic effect of an alkyl chain (a complex notion whose components are still poorly understood) is generally considered to be roughly proportional to its area in contact with water [11, 12].

It should be emphasized that fluorocarbons and *F*-alkyl chains are not only strongly hydrophobic but lipophobic as well. Therefore, they can develop a lipophobic effect on top of their hydrophobic effect. The former is clearly illustrated, for example, by the fact that certain simple mixed fluorocarbon-hydrocarbon diblock compounds of the  $\text{C}_n\text{F}_{2n+1}\text{C}_m\text{H}_{2m+1}$  type (*FnHm*, fig 3), form micelles [13] and fibrous gels [14] when dispersed in a fluorocarbon or a hydrocarbon.

#### Synthesis of fluorosurfactants

Numerous pure fluorosurfactant molecules have recently been reported in the literature (see for examples and for further references [5, 6, 15–24]). Most commercial products consist, on the other hand, of complex, often poorly defined mixtures, not well suited for physicochemical determinations or biomedical experimentation.



**Fig 3.** Mixed fluorocarbon-hydrocarbon diblock molecules utilized as components of disperse systems [35, 40–42].

As part of a program aimed at developing novel or improved fluorocarbon systems susceptible of biomedical applications, the synthesis of a variety of well-defined pure fluorosurfactants was undertaken, including neutral, anionic, cationic and zwitterionic compounds, single or double-tailed, with tails identical or not, and with a diversity of polar head groups [6, 16, 24]. A modular molecular design (fig 4) [16, 25] was elected, that allows stepwise variation in hydrophilic, lipophilic and fluorophilic characters, size and shape, chemical functions available for further derivatization, etc. The polar heads included diverse types of polyols, sugars, amino acids, amine oxides, phosphatides, polyhydroxylated telomers, etc [6, 24]. Figure 5 shows typical examples of such materials. Any desirable bioactive agent, immunological or other marker may of course be grafted onto fluorosurfactants just as they can be grafted onto standard amphiphiles.

Fluorosurfactants constitute versatile new components for the preparation of vesicles, fibers, tubules, ribbons, helices and other supramolecular aggregates. These compounds can be used to manipulate the characteristics of such systems and to modify those of standard hydrocarbon systems. Fluorosurfactants are also unique for the formulation of multi-phase, multi-component colloidal systems, including direct and reverse fluorocarbon emulsions, multiple emulsions, microemulsions, gels, aerosols, etc (fig 6), many of which express potential for drug delivery and targeting [25-31]. A parallel research objective is, from a more fun-

damental standpoint, to determine and understand the impact of the fluorinated moieties on the formation and behavior of the colloidal systems of which they are a part.

A definite prerequisite for fluorosurfactants to be used in biomedical research and in pharmaceuticals is that they be pure and well characterized. This supposes synthetic strategies and laboratory practices that integrate these requirements from the outset. Convenient starting materials to produce fluorosurfactants are available primarily in the form of perfluoroalkyl iodides,  $C_nF_{2n+1}I$  ( $R_FI$ ), and acids  $C_nF_{2n+1}COOH$ . Compounds of the  $C_nF_{2n+1}C_2H_4X$  type, with  $X = I, OH, SH, COOH$ , etc, are also commercially available and constitute valuable reagents as the  $C_2H_4$  spacer shields the  $X$  group from the influence of the  $F$ -alkyl chain. The reactivity of  $X$  is then usually back to normal, and standard synthetic procedures can be applied. Hydrophobes with longer alkyl spacers are easily obtained by adding  $R_FI$  onto appropriate terminally unsaturated alkyl chains. The synthesis and purification of fluorosurfactants can, however, be frustratingly complicated due to high surface activity and decreasing solubility that is usually observed when the length of the  $F$ -alkyl chain increases.

#### Fluorocarbon-hydrocarbon diblocks

The simple (perfluoroalkyl)alkanes 6-8 (fig 3) are amphiphiles, not in the usual sense, associating hydrophilic and lipophilic moieties, but in an extended sense in which one

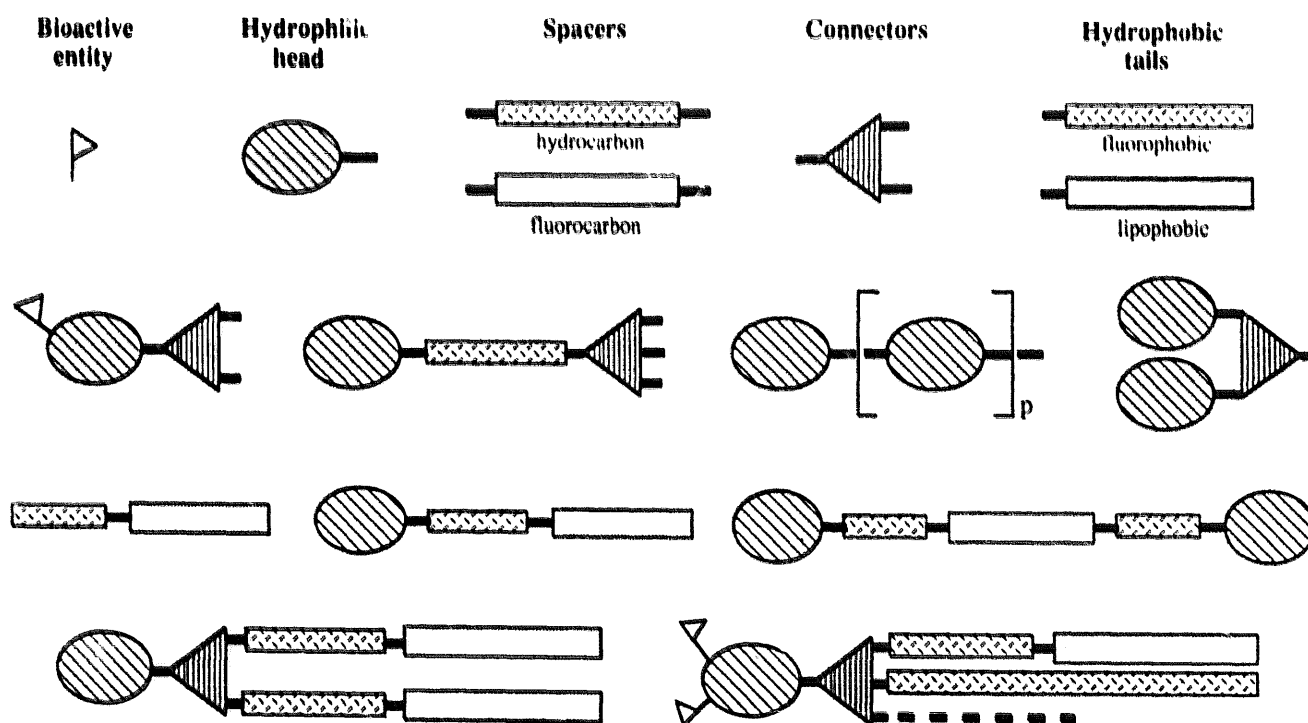
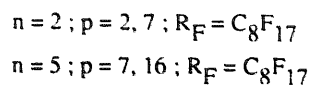
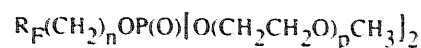
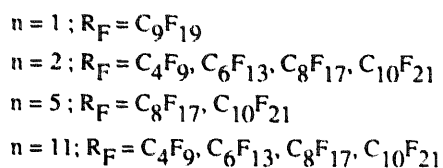
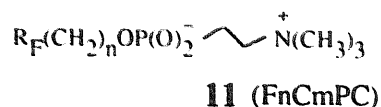
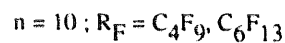
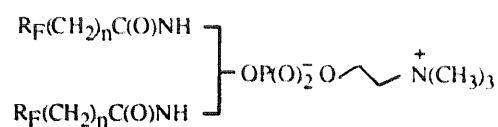
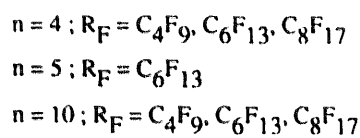
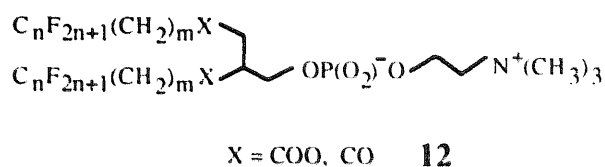
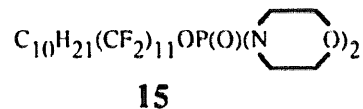
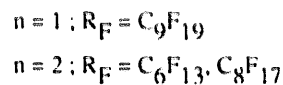
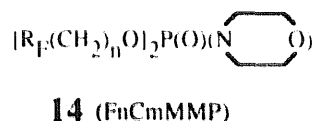
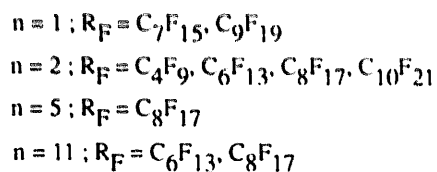
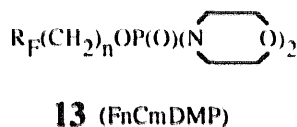
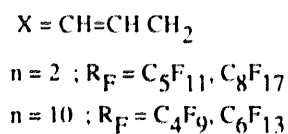
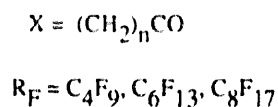
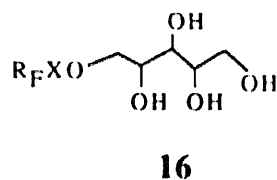


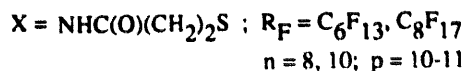
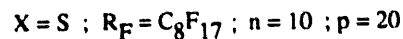
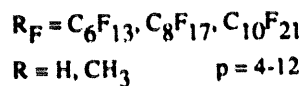
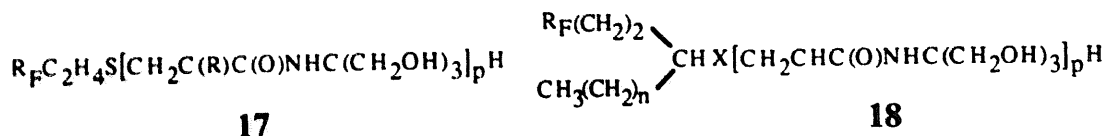
Fig 4. A modular construction set for fluorinated amphiphiles that allows stepwise adjustment of properties. From [25].



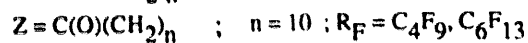
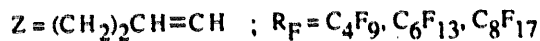
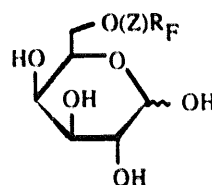
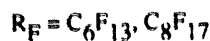
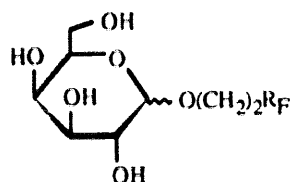
**Phosphocholine****Phosphatidylcholine****Morpholinophosphates****Xylitol**

**Fig 5.** Typical examples of recently synthesized fluorosurfactants intended for use in biomedical research and in pharmaceuticals [6, 24].

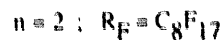
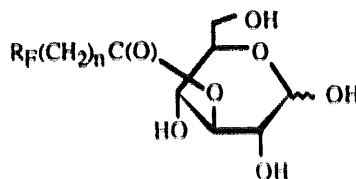
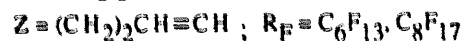
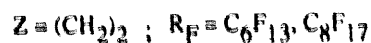
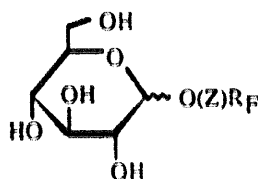
**Telomers derived from tris(hydroxymethyl)aminomethane**



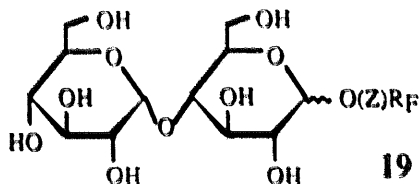
**Galactose**



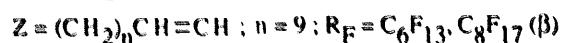
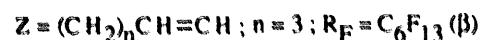
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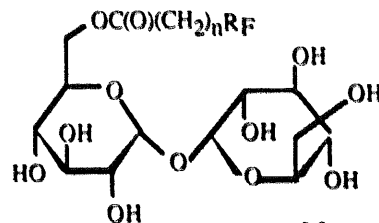
**Maltose**



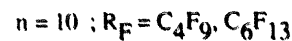
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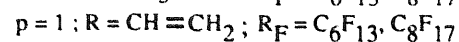
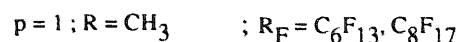
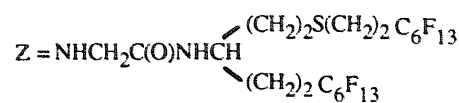
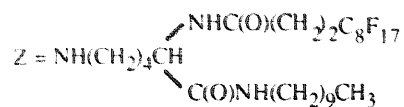
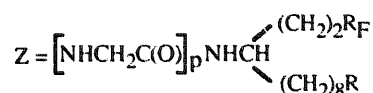
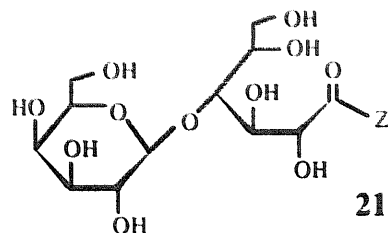
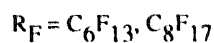
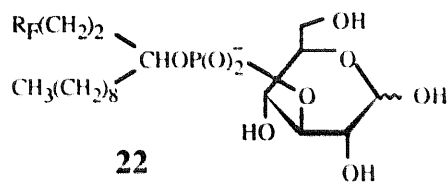
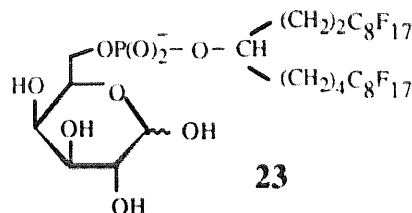
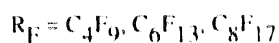
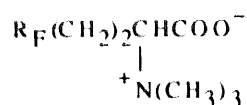
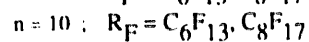
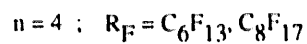
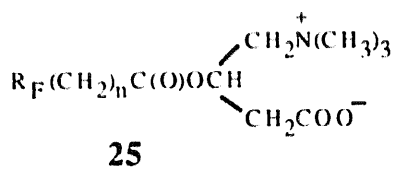
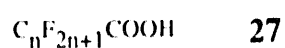
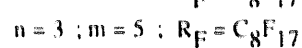
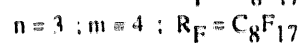
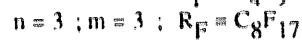
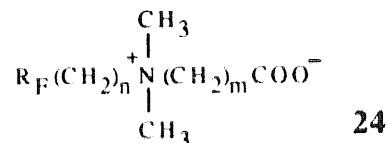
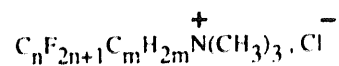
**Trehalose**



20



**Fig 5. Continued.**

**Lactose****Glucophospholipids****Galactophospholipids****Alanine****Carnitine****Acid****Betaine****Amine oxide****26****Ammonium****Fig 5. Continued.**

of the contraries is lipophilic and the other fluorophilic. The surface activity of mixed fluorocarbon-hydrocarbon diblocks is illustrated by their capacity to reduce the surface tension of hydrocarbons [32]. These diblock compounds present a strong dipole moment. They have also a dielectric constant higher than those of both the totally fluorinated and the totally hydrogenated analogs [1]. The amphiphilic character of the compounds of figure 3 is further illustrated by their capacity to self-assemble in both hydrocarbon and fluorocarbon media.  $C_8F_{17}C_{16}H_{33}$ , for example, forms micelles in toluene, and reverse micelles in perfluorooctane [13], as well as bilayer-type crystal structures [33, 34]. Bilayer-based fibers were reported to form in benzene and 2-butanone from amphiphiles 9 and 10 (fig 3), which bear two (or one) fluorocarbon chains and one (or two) hydrogenated chain grafted onto a chiral L-glutamate residue [35].

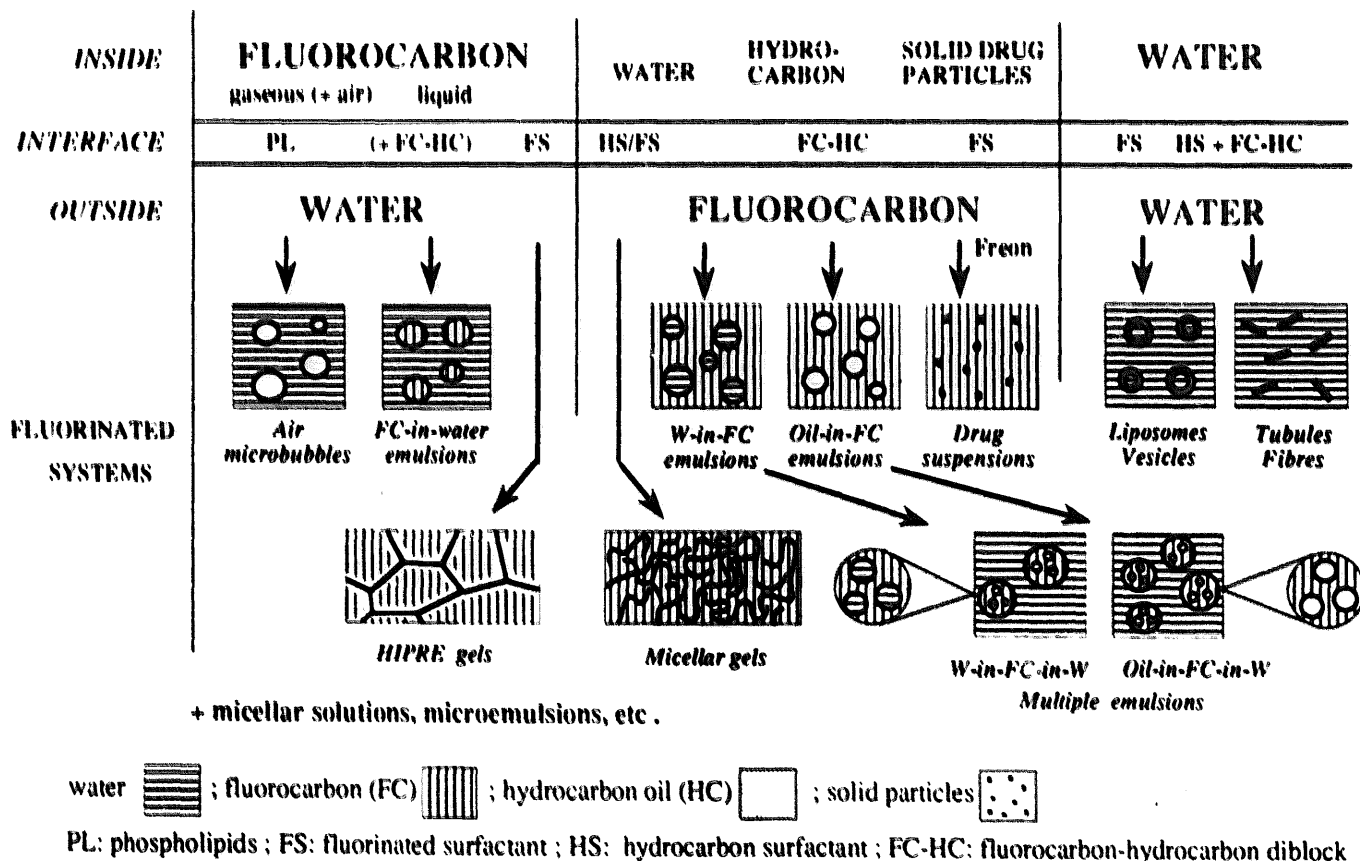
$C_nF_{2n+1}C_mH_{2m+1}$  (FnHm, 6, fig 3) diblocks can fulfill multiple functions: they were shown to strongly stabilize fluorocarbon emulsions and to allow precise control of the emulsion's particle size [36]; they also allow the preparation of lipid-in-fluorocarbon emulsions [37]; when added to the fluorocarbon phase of such emulsions they enhance the solubility of lipophilic drugs; when incorporated into liposomal membranes, they strongly increase the shelf stability of the liposomes, reduce their permeability [38], and can mod-

ify their behavior in a biological milieu [39]. The synthesis and purification of mixed fluorocarbon-hydrocarbon diblocks is straightforward [40–42].

#### *Biocompatibility and pharmacology of fluorosurfactants*

The biocompatibility of liquid fluorocarbons is well documented as a result of the intensive efforts that have been devoted to the development of injectable, 'drinkable', or 'breathable' oxygen carriers and imaging agents [26, 27, 43–51]. In contrast, research regarding the pharmacokinetics and toxicity of fluorosurfactants is still very limited.

The available data indicate that introduction of a fluorinated chain into a surfactant does generally not increase its acute toxicity, rather the opposite, despite considerably increased surface activity [6]. Intravenous LD<sub>50</sub> values in mice depend strongly on the polar head and the structure of the hydrophobic tail(s) (table 1). Values as large as 7.7 g/kg, 4 g/kg and 2 g/kg were reached for compounds of the series 12, 17 and 13, respectively. Comparison with hydrocarbon analogs shows a roughly parallel variation of LD<sub>50</sub> or MTD values. These values are usually higher for the fluorosurfactant than for its hydrocarbon analog on a weight by weight basis; this advantage is less pronounced on a mol per mol



**Fig 6.** Recently developed colloidal systems based on highly fluorinated materials.

**Table I.** Acute toxicity of some fluorosurfactants. Examples of LD<sub>50</sub> (lethal dose for half of the population treated) or MTD (maximum tolerated dose) values measured for typical fluorosurfactants and some hydrocarbon analogs (in italics) [6]<sup>a</sup>.

Compound	In vivo toxicity	
	LD <sub>50</sub>	MTD
C <sub>4</sub> F <sub>9</sub> C <sub>11</sub> H <sub>22</sub> -phosphocholine <b>11</b>		125 (8/10)
C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> -phosphocholine <b>11</b>	< 50	25
C <sub>8</sub> F <sub>17</sub> C <sub>5</sub> H <sub>10</sub> -phosphocholine <b>11</b>		125
C <sub>8</sub> F <sub>17</sub> C <sub>11</sub> H <sub>22</sub> -phosphocholine <b>11</b>		25
(C <sub>6</sub> F <sub>13</sub> C <sub>4</sub> H <sub>8</sub> ) <sub>2</sub> -glycerophosphocholine <b>12</b>		> 2800
(C <sub>8</sub> F <sub>17</sub> C <sub>4</sub> H <sub>8</sub> ) <sub>2</sub> -glycerophosphocholine <b>12</b>		> 2800
(C <sub>4</sub> F <sub>9</sub> C <sub>10</sub> H <sub>20</sub> ) <sub>2</sub> -glycerophosphocholine <b>12</b>	> 7700 (6/10)	
<i>Egg yolk phospholipids</i>	7000–10000	
C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> OP(O)[N(C <sub>2</sub> H <sub>4</sub> ) <sub>2</sub> O] <sub>2</sub> <b>13</b>		* > 2000 (10/10)
C <sub>8</sub> F <sub>17</sub> C <sub>11</sub> H <sub>22</sub> OP(O)[N(C <sub>2</sub> H <sub>4</sub> ) <sub>2</sub> O] <sub>2</sub> <b>13</b>	*4000	> 2000 (10/10)
(C <sub>6</sub> F <sub>13</sub> C <sub>2</sub> H <sub>4</sub> O) <sub>2</sub> P(O)N(C <sub>2</sub> H <sub>4</sub> ) <sub>2</sub> O <b>14</b>		* > 4000 (8/10)
(C <sub>9</sub> F <sub>19</sub> CH <sub>2</sub> O) <sub>2</sub> P(O)N(C <sub>2</sub> H <sub>4</sub> ) <sub>2</sub> O <b>14</b>		* > 2000 (9/10)
C <sub>8</sub> F <sub>17</sub> CH = CHCH <sub>2</sub> -xylitol-5 <b>16</b>	1190	460
C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> S[C <sub>2</sub> H <sub>4</sub> C(O)NHC(CH <sub>2</sub> OH) <sub>3</sub> ] <sub>6</sub> <b>17</b>	< 2350 (0/10)	> 1820 (10/10)
<i>C<sub>10</sub>H<sub>21</sub>-S[C<sub>2</sub>H<sub>4</sub>C(O)NHC(CH<sub>2</sub>OH)<sub>3</sub>]<sub>6</sub></i>	> 625 (7/10)	
C <sub>6</sub> F <sub>13</sub> C <sub>2</sub> H <sub>4</sub> S[C <sub>2</sub> H <sub>4</sub> C(O)NHC(CH <sub>2</sub> OH) <sub>3</sub> ] <sub>5</sub> <b>17</b>		3950 (9/10)
<i>C<sub>8</sub>H<sub>17</sub>-S[C<sub>2</sub>H<sub>4</sub>C(O)NHC(CH<sub>2</sub>OH)<sub>3</sub>]<sub>4</sub></i>		< 1250 (8/10)
(C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> )(C <sub>9</sub> H <sub>19</sub> )CHNHC(O)C <sub>2</sub> H <sub>4</sub> S[C <sub>2</sub> H <sub>4</sub> C(O)NHC(CH <sub>2</sub> OH) <sub>3</sub> ] <sub>14</sub>	<b>18</b>	> 3750
(C <sub>6</sub> F <sub>13</sub> C <sub>2</sub> H <sub>4</sub> )(C <sub>9</sub> H <sub>19</sub> )CHNHC(O)C <sub>2</sub> H <sub>4</sub> S[C <sub>2</sub> H <sub>4</sub> C(O)NHC(CH <sub>2</sub> OH) <sub>3</sub> ] <sub>10</sub>	<b>18</b>	2500 (9/10)
C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> -maltose-1 <b>19</b>		250
<i>C<sub>10</sub>H<sub>21</sub>-maltose-1</i>		< 125 (8/10)
C <sub>8</sub> F <sub>17</sub> C <sub>4</sub> H <sub>8</sub> C(O)-trehalose-6 <b>20</b>		250
C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> SC <sub>2</sub> H <sub>4</sub> C(O)NHC(CH <sub>2</sub> O-galactose) <sub>3</sub>	> 1300 (8/10)	> 500
<i>C<sub>12</sub>H<sub>25</sub>-SC<sub>2</sub>H<sub>4</sub>C(O)NHC(CH<sub>2</sub>O-galactose)<sub>3</sub></i>	< 500 (0/10)	> 250
C <sub>6</sub> F <sub>13</sub> C <sub>2</sub> H <sub>4</sub> OP(O) <sub>2</sub> <sup>-</sup> -glucose-6	750	
(C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> )(C <sub>8</sub> F <sub>17</sub> C <sub>4</sub> H <sub>8</sub> )CHOPO <sub>3</sub> <sup>-</sup> -galactose-6 <b>23</b>		500
(C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> )(C <sub>9</sub> H <sub>19</sub> )CHOPO <sub>3</sub> <sup>-</sup> -galactose-6	< 250 (2/10)	120
(C <sub>6</sub> F <sub>13</sub> C <sub>2</sub> H <sub>4</sub> )(C <sub>9</sub> H <sub>19</sub> )CHOPO <sub>3</sub> <sup>-</sup> -mannose-6	< 250 (4/10)	120
C <sub>4</sub> F <sub>9</sub> (CH <sub>2</sub> ) <sub>2</sub> N <sup>+</sup> (Me) <sub>2</sub> CH <sub>2</sub> C (O)O <sup>-</sup> <b>24</b>	375	250
C <sub>8</sub> F <sub>17</sub> (CH <sub>2</sub> ) <sub>3</sub> N <sup>+</sup> (Me) <sub>2</sub> (CH <sub>2</sub> ) <sub>3</sub> C (O)O <sup>-</sup> <b>24</b>	250	125
C <sub>8</sub> F <sub>17</sub> (CH <sub>2</sub> ) <sub>4</sub> C(O)-carnitine <b>25</b>		250
<i>C<sub>12</sub>H<sub>25</sub>C(O)-carnitine</i>	< 125	

<sup>a</sup>iv (tail vein) or \*ip in mice (n = 10).

basis. One should, however, keep in mind that, because of their high efficiency, fluorosurfactants can often be used in concentrations 10 to 100 times smaller than those used for classical surfactants.

Perfluoroalkanoic acids, **27**, have received particular attention due to their industrial applications as catalysts in certain polymerization reactions. These acids were found to be rather toxic, with ip LD<sub>50</sub> values of 190 and 40 mg/kg body weight in rat for perfluorooctanoic (PFOA) and perfluorodecanoic (PFDA) acids, respectively [52]. The patterns of toxicities observed appear to be significantly different for these two acids, and are also species- and sex-dependent. Both acids induced peroxisome proliferation in hepatocytes and considerable changes in enzyme activities.

No metabolites were identified. On the other hand, no ill effects were reported in plant workers exposed to PFOA in spite of the considerably higher than normal organic fluorine levels found in their blood [53]. The distribution, metabolism and excretion of PFOA and PFDA have been investigated rather extensively [54, 55].

Other, more limited biodistribution and disposition studies involve the nonionic telomer **17** (R<sub>F</sub> = C<sub>6</sub>F<sub>13</sub>, P = 5–6) [56]. Rats were given a 100 mg/kg dose of CO (amide)-[<sup>14</sup>C]-labeled telomer by the iv or ip routes. The compound was found in all organs except the brain. The radioactivity was mainly excreted with the urine. Some <sup>14</sup>CO<sub>2</sub> was found in the expired air. Urine and plasma analysis with an anionic resin showed that part of the telomer had converted into a

polyanionic form, indicating that metabolism had occurred, possibly by hydrolysis of the amide bond. There was no evidence, however, that the *F*-alkyl chain was metabolized.

Hemolytic activity is strongly reduced and often suppressed when fluorinated chains are introduced in a surfactant [28, 57]. For example, no detectable hemolysis was seen with a 30 gL<sup>-1</sup> micellar solution of **13** (F8C2DMP), while its hydrogenated analog is highly hemolytic at 1 gL<sup>-1</sup>. Hemolysis by fluorosurfactants is actually seen to decrease as fluorinated chain length, and consequently surface activity, increase within a series. The longer the fluorocarbon chain, the less hemolytic the compound (table II). This is

the exact opposite of what usually happens with hydrocarbon surfactants.

Some attention was given to fluorocarbon-hydrocarbon diblock amphiphiles. No effect on the growth and viability was found when such compounds were incubated with Namalva cells [36]. No inhibition of carcinoma cell proliferation was noted with C<sub>6</sub>F<sub>13</sub>C<sub>10</sub>H<sub>21</sub>, while proliferation decreased by 80% in the presence of decane [58]. The growth and survival of mice was not affected by the intraperitoneal administration of about 30 g/kg body weight doses of C<sub>6</sub>F<sub>13</sub>CH=CHC<sub>10</sub>H<sub>21</sub> (F6H10E) or of F6H10 [36, 59]. Even the iodinated mixed compound C<sub>6</sub>F<sub>13</sub>CH=CHC<sub>6</sub>H<sub>13</sub> was tolerated intraperitoneally by mice at a 45 g/kg body weight dose [60].

**Table II.** Hemolytic activity of fluorinated surfactants as compared to hydrocarbon analogs [6, 28, 57].

Compound	Hemolytic activity		
	g L <sup>-1</sup>	mmol L <sup>-1</sup>	Grading <sup>a</sup>
C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> -phosphocholine <b>11</b>	30	47.7	0
C <sub>8</sub> F <sub>17</sub> C <sub>5</sub> H <sub>10</sub> -phosphocholine <b>11</b>	50	74.5	0
C <sub>8</sub> F <sub>17</sub> C <sub>11</sub> H <sub>22</sub> -phosphocholine <b>11</b>	1	1.32	+++
C <sub>10</sub> H <sub>21</sub> -phosphocholine	1	1.55	++++
C <sub>16</sub> H <sub>33</sub> -phosphocholine	0.0025	0.006	+++
(C <sub>6</sub> F <sub>13</sub> C <sub>4</sub> H <sub>8</sub> ) <sub>2</sub> -glycerophosphocholine <b>12</b>	60	62.4	0
(C <sub>8</sub> F <sub>17</sub> C <sub>4</sub> H <sub>8</sub> ) <sub>2</sub> -glycerophosphocholine <b>12</b>	60	56.6	0
(C <sub>4</sub> F <sub>9</sub> C <sub>10</sub> H <sub>20</sub> ) <sub>2</sub> -glycerophosphocholine <b>12</b>	100	105	0
(C <sub>9</sub> H <sub>19</sub> ) <sub>2</sub> -glycerophosphocholine	0.75	1.33	++++
C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> OP(O)[N(C <sub>2</sub> H <sub>4</sub> ) <sub>2</sub> O] <sub>2</sub> <b>13</b>	30	44	0
C <sub>10</sub> H <sub>21</sub> OP(O)[N(C <sub>2</sub> H <sub>4</sub> ) <sub>2</sub> O] <sub>2</sub>	1	1.33	++++
C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> S[C <sub>2</sub> H <sub>4</sub> C(O)NHC(CH <sub>2</sub> OH) <sub>3</sub> ] <sub>6</sub> <b>17</b>	200		0
C <sub>10</sub> F <sub>21</sub> C <sub>2</sub> H <sub>4</sub> S[C <sub>2</sub> H <sub>4</sub> C(O)NHC(CH <sub>2</sub> OH) <sub>3</sub> ] <sub>6</sub> <b>17</b>	200		0
C <sub>10</sub> H <sub>21</sub> -S[C <sub>2</sub> H <sub>4</sub> C(O)NHC(CH <sub>2</sub> OH) <sub>3</sub> ] <sub>6</sub>	1		++++
C <sub>6</sub> F <sub>13</sub> C <sub>2</sub> H <sub>4</sub> -maltose-1 <b>19</b>	100		0
C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> -maltose-1 <b>19</b>	40 <sup>b</sup>		0
C <sub>10</sub> H <sub>21</sub> -maltose-1	1		++
C <sub>6</sub> F <sub>13</sub> C <sub>4</sub> H <sub>8</sub> C(O)-trehalose-6 <b>20</b>	50		0
C <sub>8</sub> F <sub>17</sub> C <sub>4</sub> H <sub>8</sub> C(O)-trehalose-6 <b>20</b>	30 <sup>b</sup>		0
C <sub>4</sub> F <sub>9</sub> C <sub>10</sub> H <sub>20</sub> C(O)-trehalose-6 <b>20</b>	0.1		++++
C <sub>10</sub> H <sub>21</sub> C(O)-trehalose-6	1		++++
C <sub>6</sub> F <sub>13</sub> C <sub>2</sub> H <sub>4</sub> OP(O) <sub>2</sub> <sup>-</sup> -glucose-6	100		0
C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>4</sub> OP(O) <sub>2</sub> <sup>-</sup> -glucose-6	30 <sup>b</sup>		0
C <sub>10</sub> H <sub>21</sub> OP(O) <sub>2</sub> <sup>-</sup> -glucose-6	5		++++
C <sub>8</sub> F <sub>17</sub> (CH <sub>2</sub> ) <sub>3</sub> N <sup>+</sup> (Me) <sub>2</sub> (CH <sub>2</sub> ) <sub>3</sub> C(O)O <sup>-</sup> <b>24</b>	200	358	0
C <sub>12</sub> H <sub>25</sub> N <sup>+</sup> (Me) <sub>2</sub> (CH <sub>2</sub> ) <sub>3</sub> C(O)O <sup>-</sup>	1	3.25	+++
C <sub>8</sub> F <sub>17</sub> (CH <sub>2</sub> ) <sub>4</sub> C(O)-carnitine <b>25</b>	25		0
C <sub>6</sub> F <sub>13</sub> (CH <sub>2</sub> ) <sub>10</sub> C(O)-carnitine <b>25</b>	0.1		++++
C <sub>10</sub> H <sub>21</sub> C(O)-carnitine	2		++++

<sup>a</sup>Grading: 0, +, ++, +++, +++++ correspond to percentages of hemolysis between 0 and 5%, 5 and 15, 15 and 40, 40 and 60, and > 60%, respectively; 0.9% NaCl and distilled water produce 0 and 55% hemolysis, respectively. <sup>b</sup>After dispersion in a Pluronic F-68 solution.

The biodistribution and excretion of the diblock compound F6H10E, was investigated in rats using a 25% w/v emulsion of pure diblock [36, 59]. The animals received a 3.6 g/kg dose of the compound intravenously; tissue distribution was assessed by  $^{19}\text{F}$  NMR. A half-life of  $25 \pm 5$  days in the liver, the organ which had taken up most (70%) of the injected dose, was estimated for F6H10E. No new signals were detected in the  $^{19}\text{F}$  spectra, indicating that metabolism was absent or minimal, which, if confirmed by more sensitive techniques, should greatly simplify further toxicology and pharmacology studies. The shorter diblock F8H8 was determined to have an organ half-life of about 14 days (Weers JG, personal communication).

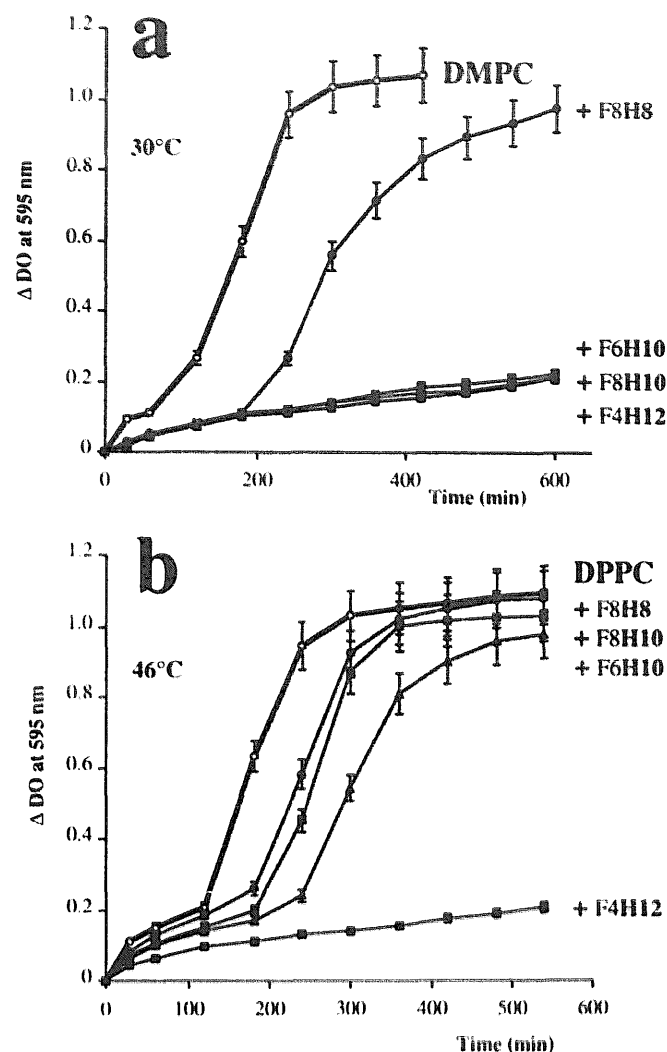
#### *Impact of fluorosurfactants on the behavior of particles in a biological milieu*

The ability of fluorosurfactants to influence the *in vitro* adsorption of proteins and phagocytic uptake of particles was investigated using calibrated polystyrene microspheres. The microspheres were coated with the surfactant and incubated in swine serum or submitted to phagocytosis by mouse peritoneal macrophages. Both the fluorinated chain and the hydrocarbon spacer length had an effect. Reduced protein adsorption and phagocytic uptake in serum was observed when the microspheres were, for example, coated with  $\text{C}_8\text{F}_{17}\text{C}_m\text{H}_{2m}\text{PC}$ , **11** ( $m = 2$  and  $5$ ), uptake was then comparable to that observed with a pegylated phospholipid. No effect was seen with a longer spacer, as in **11** ( $n = 8$ ,  $m = 11$ ), or with non-fluorinated analogs [61]. Lower phagocytic uptake was also observed when the microspheres were coated with certain of the tris(hydroxymethyl)amino-methane-derived telomers **17** [62].

Drastic reduction of the rate of enzymatic hydrolysis of phospholipids by pancreatic phospholipase  $\text{A}_2$  was observed when diblocks of type **6** were introduced in the membrane of a liposome [39]. In order for the phenomenon to be observed, the length of the hydrocarbon moiety of the diblock must be sharply adjusted to that of the phospholipid's fatty acid chains (fig 7). It must have at least ten carbon atoms in the case of DMPC and twelve in the case of DPPC, indicating that this moiety is probably inserted among the fatty acid chains of these compounds. The length of the fluorinated moiety does not appear to be determining but the presence of the fluorinated chain is indispensable, which again illustrates its role as a membrane-structuring element.

The intravascular persistence in mice of carboxyfluorescein-loaded vesicles made of fluorinated phospholipid of type **12** was several times larger than those of similarly sized conventional DSPC or DSPC/cholesterol liposomes [63]. As in the case of 'pegylated' liposomes, the circulation time was independent of the dose administered.

It is certainly remarkable that the internal structure of a fluorinated film can have such impact on the behavior of particles *in vivo* or in a biological milieu.

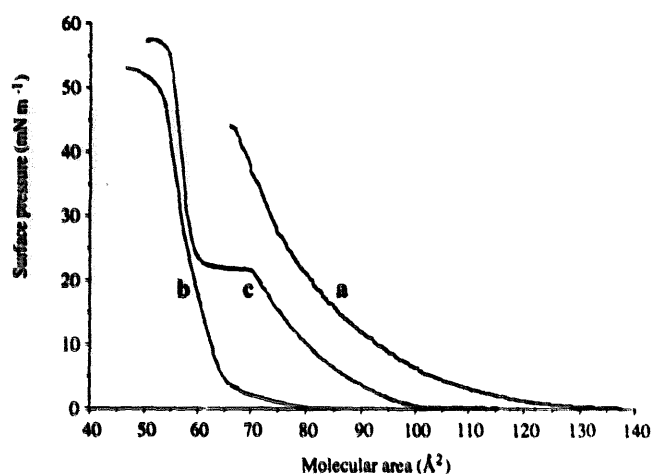


**Fig 7.** Incorporating fluorocarbon-hydrocarbon diblocks,  $\text{C}_n\text{F}_{2n+1}\text{C}_m\text{H}_{2m+1}$  (FnHm), into vesicles made from DMPC (dimyristoylphosphatidylcholine) (a) or DPPC (dipalmitoylphosphatidylcholine) (b) results in a drastic reduction of the rate of enzymatic hydrolysis of these phospholipids by pancreatic phospholipase  $\text{A}_2$  (as monitored by changes of absorbance of a pH indicator). From [39].

#### **Self-aggregation of fluorinated amphiphiles**

The greater hydrophobic effect developed by *F*-alkyl chains as compared to alkyl chains, combined with the above mentioned lipophobic effect, and the larger cross section and higher rigidity of the chains, result in an enhanced tendency for fluorinated amphiphiles to self-assemble. Sturdy mono and bilayers, and diverse lamellar and non-lamellar phases form at low surfactant concentrations, in water and other solvents.





**Fig 8.** Compression isotherms of Langmuir monolayers made from fluorinated double-chain monomorpholinophosphates **14** (FnCmMMP, fig 5). F6C2MMP forms a liquid monolayer (a), F9C1MMP a highly organized solid monolayer (b), and F8C2MMP presents a liquid-to-solid transition (c). This indicates that increasing the degree of fluorination of the tail, while maintaining its total length constant, leads to increasing order, and that subtle changes in chain length can induce substantial modifications in the monolayer's behavior. From [67].

#### Langmuir films and black lipid membranes

Fluorinated amphiphiles readily form Langmuir monolayers [64]. The higher difference in energy between gauche and trans conformations, and the reduced number of kinks present in fluorinated vs hydrogenated chains facilitate the amphiphile's organization. Fluorinated chains have a strong tendency to orient themselves perpendicularly to the water surface due to exceedingly low chain-water interactions. This is illustrated by the fact that even a non-polar molecule, perfluoro-*n*-eicosane, C<sub>20</sub>F<sub>42</sub>, can form ordered and stable monolayers at the surface of water [65].

Further studies of monolayers made from fluorinated amphiphiles include those from Elbert *et al* [9], Barton *et al* [2] and Jacquemain *et al* [66]. Monolayers made from the single-chain phosphocholine (FnCmPC) and dimorpholinophosphate (FnCmDMP) derivatives **11** and **13** are remarkably stable, even with only ten-carbon-long hydrophobic chains such as in F9C1PC and F8C2DMP [67]. The hydrocarbon spacer inserted between the fluorinated termination and the polar head can have a definite disorganizing effect on the monolayer. In the F10CmPC series, for example, the disorder resulting from the confinement of the fluorinated and hydrocarbon segments in the same chain, as expressed by an increase of the limiting polar head area (*ie* area extrapolated at zero pressure), was found to increase when the hydrocarbon spacer increased from  $m = 2$  to 5 ( $A_{\infty} = 60 \pm 0.5$  and  $70 \pm 0.5$  Å<sup>2</sup> for F10C2PC and F10C5PC, respectively). On the other hand, the limiting area was found to

decrease for  $m = 11$  ( $A_{\infty} = 60 \pm 0.5$  Å<sup>2</sup> for F10C11PC), indicating that when the hydrocarbon spacer has reached a critical length, which was found to be about  $m = 5-7$ , chain-chain interactions may become strong enough to compensate for any induced disorder [67].

Recently, amphiphiles of type **15**, in which a *F*-alkyl segment is inserted between a hydrocarbon chain and the dimorpholinophosphate polar head, have been synthesized [68]. The study of the monolayer and self-aggregation behaviors of such 'reverse' amphiphiles is presently investigated and compared to that of 'direct' FnCmDMP amphiphiles.

Small incremental increases in fluorinated chain length can result in drastic modifications in monolayer organization and behavior. Thus, within the series of double-tailed *F*-alkylated monomorpholinophosphates **14**, F6C2MMP forms a liquid monolayer, while F8C2MMP presents an isotherm with a liquid-to-solid transition (fig 8). Increasing the Fn/Cm ratio, *ie* the degree of fluorination of the tail, while maintaining its total length constant, leads to increasing order as illustrated by the fact that F9C1MMP forms a highly organized solid monolayer, while F8C2MMP exhibits a phase transition (Goldmann *et al*, in preparation). The liquid condensed phase of both compounds is strongly organized and exhibits intense Bragg peaks when examined by grazing incidence X-ray diffraction.

Black lipid membranes (BLMs) with a perfluorinated internal film have been produced from combinations of phospholipids and fluorocarbon/hydrocarbon diblocks (Krafft *et al*, in preparation). These fluorinated BLMs turned out to be exceptionally long-lived and sturdy. Their capacitances are two to three times larger (depending on the diblock) than in the absence of diblock, and are among the highest reported to date. The thickness of the inner fluorinated core of the membrane was estimated to 20 Å in the case of F6H10 (**6**, fig 3), indicating that the fluorinated chains are not interdigitated.

#### Vesicles with a fluorinated bilayer membrane

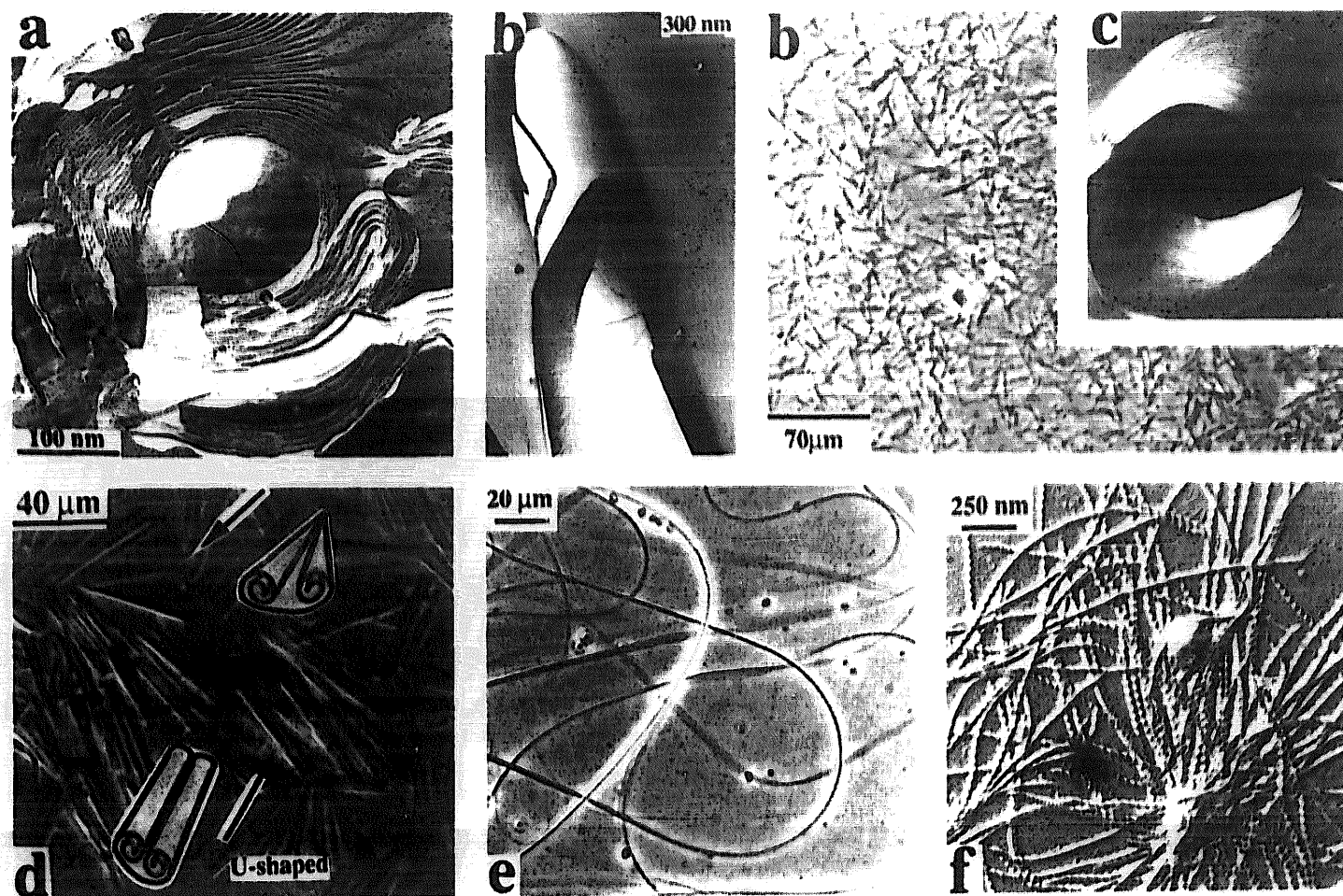
The outstanding ordering and stabilizing capacity of fluorinated amphiphiles is clearly illustrated by the finding that even short single-chain *F*-alkylated phosphocholine derivatives of type **11**, upon gentle stirring, form highly stable vesicles without the need for any co-surfactant, or for any supplementary intermolecular associative interactions such as those that derive from the presence of hydrogen bonding between polar heads, ion-pairing, polymerization, diacetylenic rod-like segments, etc [69]. These vesicles even withstand heat sterilization without noticeable changes in particle size, *ie* are more stable than those obtained from standard double-tailed phospholipids. Under the same conditions, the hydrocarbon analogs of **11** ( $n = 2$ ,  $m = 8$ ), in terms of chain length (C10PC) or in terms of equivalent hydrophobicity (C15PC), only provided micelles or 3D crystals, respectively.

It is also remarkable that certain single-chain fluorosurfactants were seen to yield a diversity of self-assemblies, including small unilamellar vesicles (SUVs), multilamellar vesicles (MLVs), giant vesicles, flexible fibers and rigid tubules, whose formation and interconversion can be controlled to a certain extent by modifying the experimental conditions (fig 9) [70].

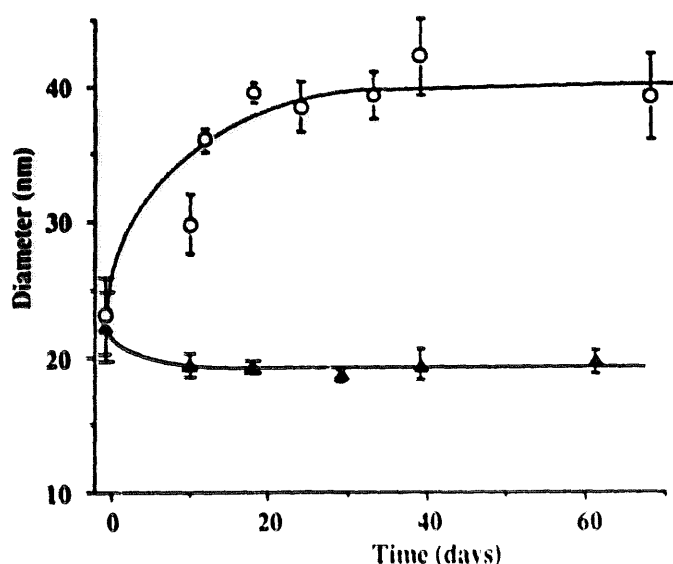
The liposomes made from the *F*-alkylated phospholipids **12** were extensively investigated. Sterilization was achieved at 120°C in standard conditions [71]. Higher gel-to-fluid transition temperatures, as compared to the hydrocarbon analogs, indicate increased ordering of the bilayer membrane [72]. Other stable, heat sterilizable fluorinated vesicles include those obtained from further examples of *F*-alkylated phospholipids [73], from various glycolipids **21** [74, 75] and glycopospholipids **23** [76]. In the latter case, the leakage rate of entrapped carboxyfluorescein was shown to depend strongly on the sugar (glucose > mannose > galactose) and decreased with increasing fluorination.

Spontaneously forming stable vesicles were recently obtained from mixtures of a perfluoropolyether surfactant and an alkylbetaine [77]. These vesicles were shown to have potential as carriers for porphyrin-based biological or biomimetic molecules.

Vesicles made from fluorinated amphiphiles are characterized by the presence of a strongly organized, adjustable fluorinated film within their bilayer membranes (fig 2a). This hydro- and lipo-phobic fluorinated membrane core confers added drug encapsulation stability as compared to hydrogenated analogs, whether in buffer or in serum [78]. The penetration/solubilization of the paramagnetic probe Tempo in the membrane of vesicles made from *F*-alkylated phospholipids **12** was shown to be suppressed when the fluorinated segment was more than four carbon atoms long [72]. The release of carboxyfluorescein entrapped in such vesicles can be significantly slowed [78]. The fluorinated membrane did not, however, prevent the active encapsulation of doxorubicin using pH or ammonium sulfate gradients [79]. The encapsulation sta-

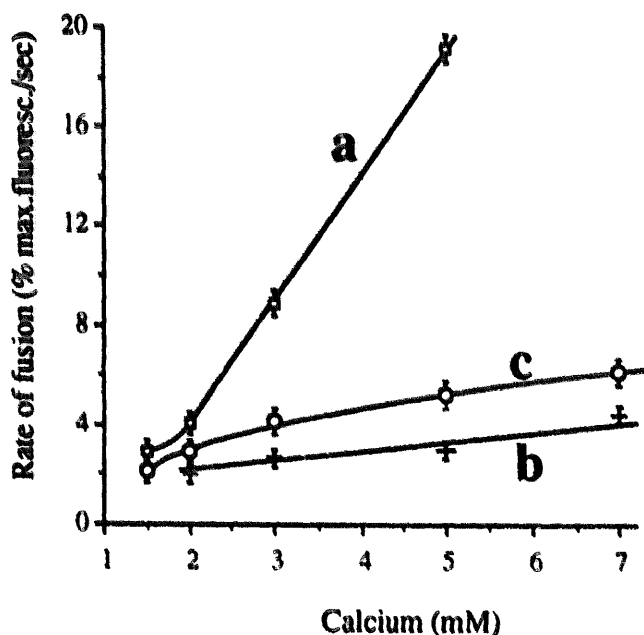


**Fig 9.** Fluorinated single-chain amphiphiles can yield a variety of supramolecular assemblies with diverse morphologies, while their non-fluorinated analogs only form micelles. **a.** Multilayer vesicles. **b.** Tubules. **c.** Internal aqueous core of a tubule. **d.** Twin tubules. **e.** Very long flexible tubules, all made from dimorpholinophosphates **13** (FnCmDMP). **f.** Helices made from a glycolipid of type **21**. From [26].

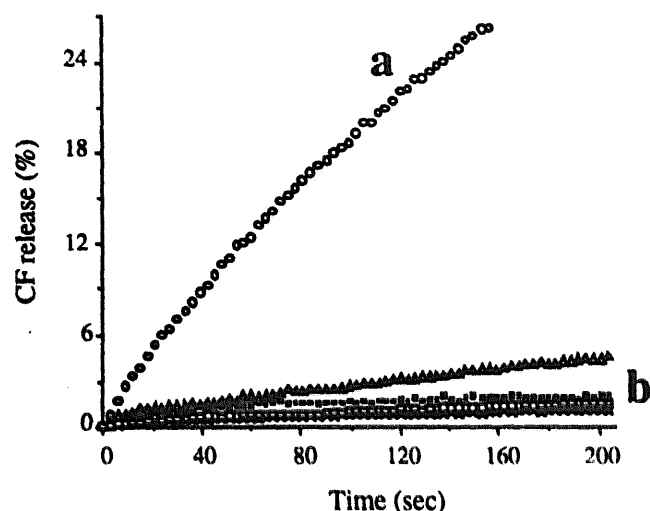


**Fig 10.** Evolution of DMPC and DMPC/F4H10E ( $C_4F_9CH=CHC_{10}H_{21}$ ) (1:2) liposomes as a function of time:  $\circ$ , DMPC;  $\Delta$ , F4H10E/DMPC. From [38].

bility of the drug was low, presumably because of high permeability to  $Na^+$  ions, which, after exchange with  $H^+$ , leads to an increase of the intraliposomal pH, thus favoring the release of the drug in its more diffusible neutral form.



**Fig 11.** Initial rate of fusion (as monitored by the terbium/dipicolinic acid assay) of phosphatidylserine (PS) SUVs (a), PS/F4H10 (1:1) SUVs (b), and PS/F6H10 (1:1) SUVs (c). Vesicle fusion was induced by adding various concentrations of  $Ca^{2+}$ . The final lipid concentration was 100  $\mu M$ . From [82].

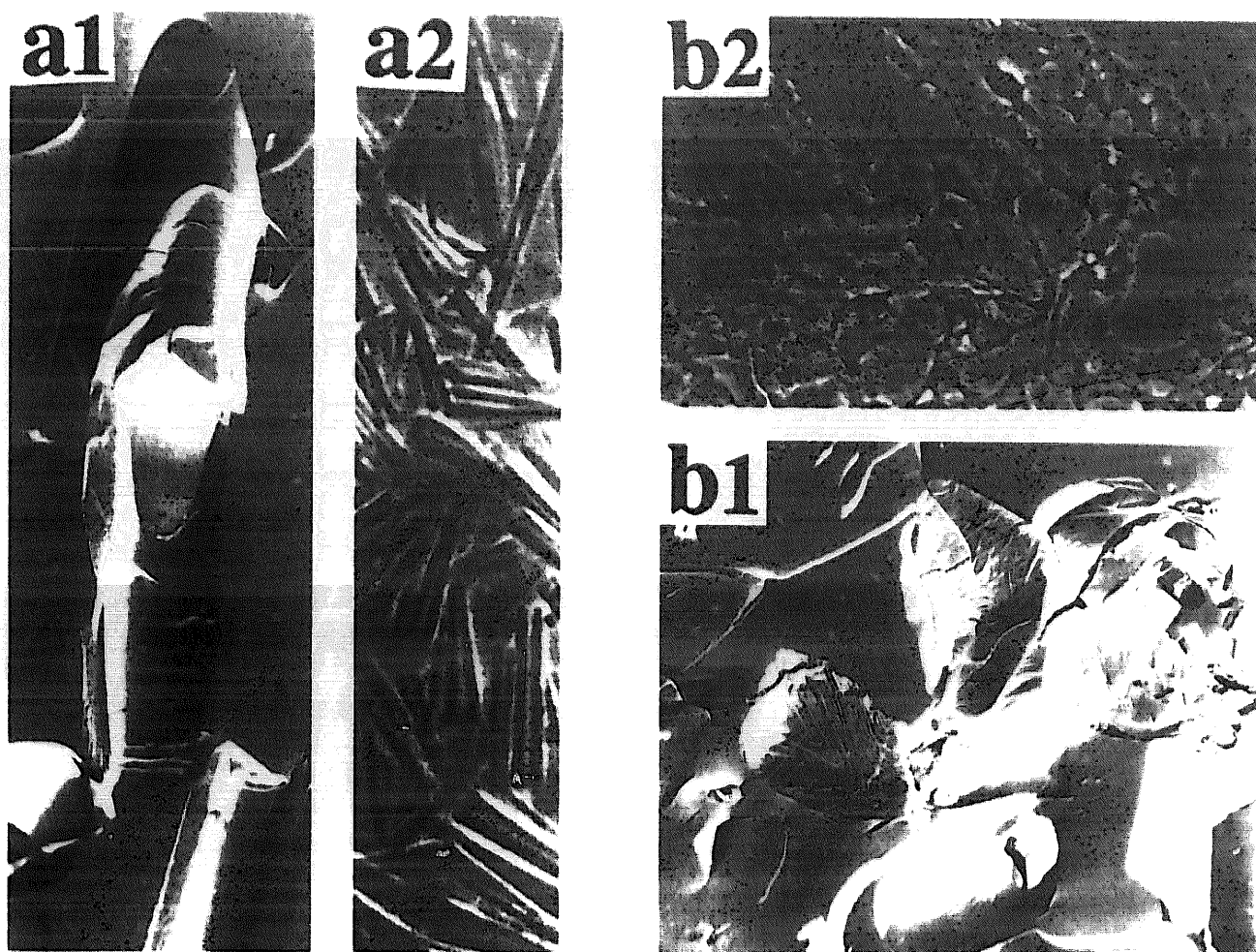


**Fig 12.** Comparative kinetics of 5,6-carboxyfluorescein (CF) release from phosphatidylserine (PS) SUVs (a) and PS/FnHm SUVs (b). 100% release corresponds to the fluorescence intensity measured after addition of Triton X-100 (0.1% v/v). The lipid concentration was 100 mM and the final  $Ca^{2+}$  concentration was 5.0 mM.  $\circ$ , pure PS SUVs;  $\times$ , F4H10/PS (1:1);  $\square$ , F4H10/PS (2:1);  $\blacksquare$ , F6H10/PS (1:1);  $\Delta$ , F8H10/PS (1:1). From [82].

Fluorinated vesicles were also obtained, at lesser expense in time and cost, by combining standard phospholipids with simple fluorocarbon-hydrocarbon diblocks such as **6** or **7** (fig 2b). Significantly greater stability and lower membrane permeability were again observed, indicating that the fluorinated segments segregate and organize tightly within the bilayer [38, 80]. Thus, vesicles made of dimyristoylphosphatidylcholine (DMPC) became heat sterilizable when two equivalents of  $C_4F_9CH=CHC_{10}H_{21}$  were incorporated into their membrane; the particle size of these vesicles had not changed noticeably during sterilization or after 2 months storage at room temperature (fig 10).

The permeability of vesicles made with the single-chain phosphocholine **11** ( $n=8$ ,  $m=2$ ) was substantially reduced when diblocks of type **6** were added [81]. In this case the hydrophobicity-driven aggregation of the fluorocarbon segments of the two components results in the reconstitution of a pseudo double-tail amphiphile and in tighter packing of the membrane.

Incorporation of FnHm semi-fluorinated alkanes (**6**,  $n=4, 6, 8$  and  $m=10$ ) in the membrane of SUVs made of phosphatidylserine also had an impact on the fusion kinetics of these SUVs. Fusion was induced by  $Ca^{2+}$  ions and monitored by the terbium/dipicolinic acid assay [82]. Both the rates of fusion (fig 11) and of release of the internal content of the vesicle (fig 12), as evaluated by the release of 5,6-carboxyfluorescein, were much lower for the fluorinated SUVs than for SUVs made of phosphatidylserine alone. The FnHm molecules may be dual acting by creating the internal



**Fig 13.** Tubule-vesicle interconversion. Tubules made from a mixture of single-chain dimorpholinophosphates **13** (F10C2DMP/F8C5DMP (1:1) (**a1**, freeze-fracture electron and **a2**, optical microscopies) transform into vesicles (in the gel state) (**b1**, freeze-fracture electron and **b2**, optical microscopies) when temperature is increased. Conversion is total at *ca* 40°C (Krafft *et al.*, in preparation).

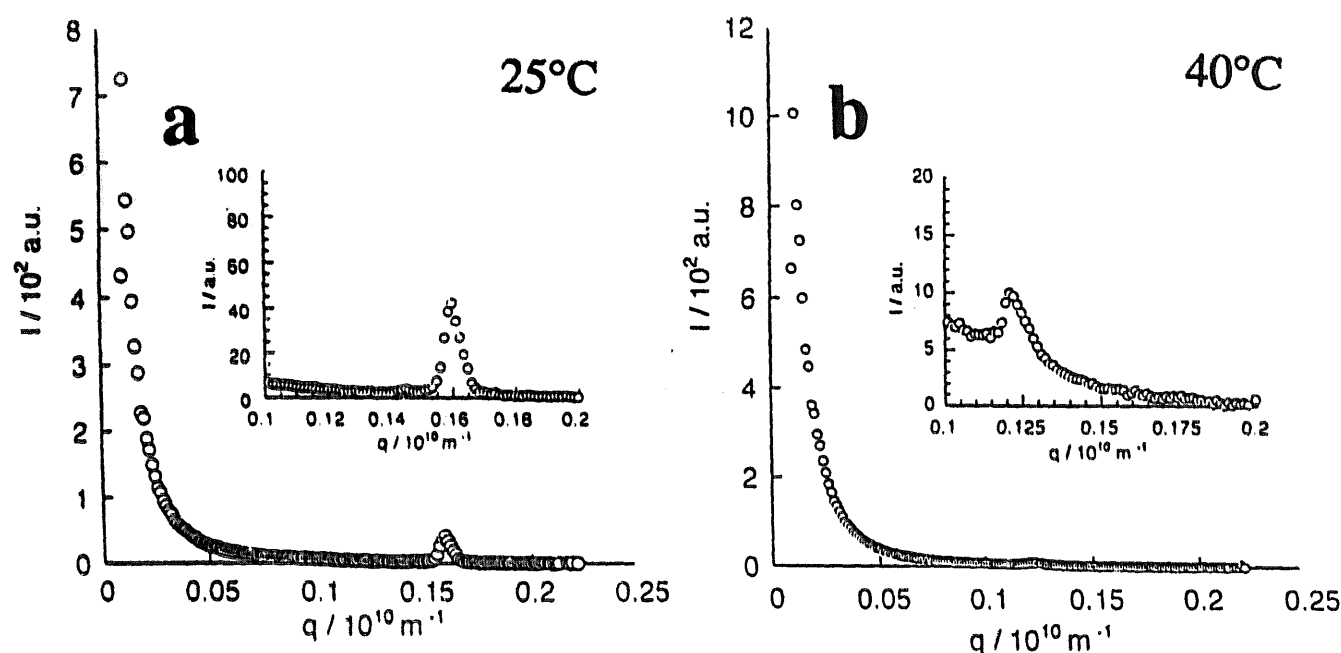
hydrophobic/lipophobic fluorinated film within the membrane, and by enhancing the van der Waals interactions in the hydrocarbon regions.

Polymerized fluorinated vesicles can be obtained, either by using polymerizable fluorinated amphiphiles to prepare the vesicles [83–85], or by incorporating a monomer in the lipophilic zones of a membrane made of fluorinated amphiphiles (Nakache *et al.*, in preparation). In the latter case polymerization results in the formation of a double shell of polymer separated by a fluorinated film. The surfactant can subsequently be removed, leaving the intact double shell, further exemplifying the use that can be made of fluorinated amphiphiles for the microstructuration of space.

### Microtubules

Hollow cylindrical microtubules made of rolled-up bilayers of amphiphiles have recently received considerable attention. The possible applications of these microtubules ranges from

use as templates for the elaboration of new components for microelectronics such as vacuum field emission cathodes, or as models of natural tubules and of enzyme clefts, to microcontainers for the controlled release of active agents [86, 87]. Microtubules have been obtained from diverse dispersions of diacetylenic phospholipids [86], aldonamides [87], glutamates [88], amino acids [89], conjugated phospholipid nucleosides [90] and glycolipids [91, 92]. Cochleate lipid cylinders form when  $\text{Ca}^{2+}$  ions are added to phosphatidylserine SUVs [93]. Tubules made from a biotinylated dioctadecylamine lipid were shown to exhibit properties of molecular recognition toward the protein streptavidin which assembles spontaneously into ordered helical arrays at the tube's surface [94]. The amphiphiles utilized for the preparation of tubules all have in common the presence of a chiral center and often also the capacity of forming hydrogen bonds between their polar heads. The chiral center was deemed necessary for the inception of the rolling-up process which leads to the tubular structures.



**Fig 14.** Small angle X-ray diffraction monitoring of the conversion of tubules (at 25°C) into vesicles (at 40°C) in dispersions of a mixture of single-chain dimorpholinophosphates **13** (F10C2DMP/F8C5DMP, 1:1). The repeating distance within bilayers is smaller in tubules (39 Å) than in vesicles (52 Å). From [96].

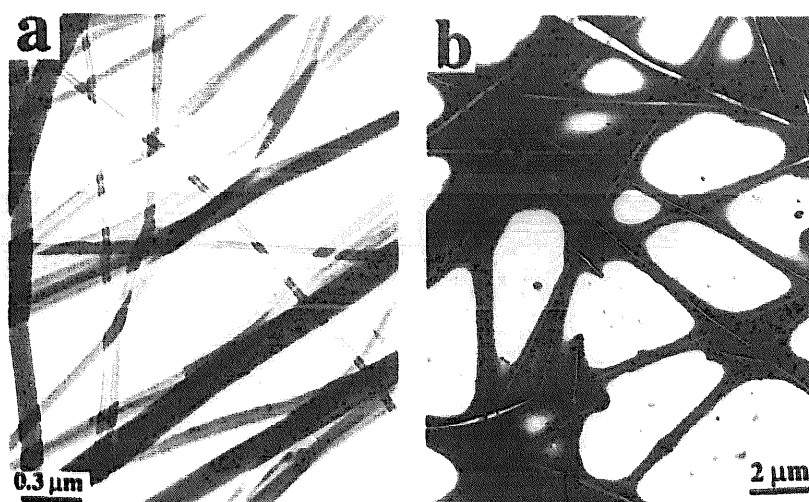
We found that microtubules could also be obtained from non-chiral, non-hydrogen bond, single-chain fluorinated amphiphiles such as **13** (fig 9) [95]. The aqueous internal channel visible on the electron micrographs was confirmed by epifluorescence microscopy after carboxyfluorescein had been incorporated. The fact that hydrocarbon analogs of **13** only form micelles further demonstrates the powerful structuring effect of the fluorinated moieties.

Tubules are generally considered as being more organized than vesicles. The tubules made of **13** actually convert into vesicles when heated (fig 13). Small angle X-ray diffraction measurements showed that the repeating distance in the bilayers was smaller in the microtubules (visualized by optical and electron microscopies at 25°C) than in the vesicles which had formed at 40°C (fig 14) [96]. The latter slowly convert back into tubules upon cooling. The tubules of **13** are exceptionally sturdy; they can be centrifuged, dried, stored, and resuspended in a solvent without noticeable alteration of their morphology. Changes in the amphiphile's molecular structure or in the tubules' preparation conditions allow adjustment of tubule length and diameter in a reproducible fashion (Giulieri *et al.*, in preparation). Fluorinated tubules were also grown in organic solvents such as dimethylsulfoxide and dimethylformamide [97]. Other tubular self-aggregates have been made by co-dispersing a *F*-alkylated phosphocholine of type **11** with a *F*-alkylated alcohol such as  $\text{C}_8\text{F}_{17}\text{C}_2\text{H}_4\text{OH}$  [98].

The mechanism of formation and evolution of tubules has been unraveled using optical and electron microscopy. In the case of tubules of F10C2DMP, monitoring in real time the various steps of their formation was possible in dimethylformamide (Giulieri *et al.*, in preparation). Formation of tubules was seen to involve the aggregation and fusion of vesicles to yield flat multibilayer sheets; these sheets were then observed to roll-up from opposite sides to form twin cylindrical microstructures, which eventually separate into isolated tubules. Depending on the amphiphile, tubules retain their morphology for many months, or collapse and expel the solvent present in the inner core, eventually yielding crystalline needles [98].

Additional tubules were obtained from anionic double-tailed glucolipids of type **22** (fig 15) [99]. The formation of these tubules is pH-dependent, indicating that the formation of hydrogen bonds between the phosphate group and a hydroxyl group of the sugar is likely to be involved. At a high pH, the polar heads of the amphiphiles are less hydrated and tubule formation is favored over vesicle formation. Both the partly-fluorinated glucophospholipid **22** and its fully hydrogenated analog form tubules. Introducing one fluorinated chain allows the vesicle-tubule transition to occur above room temperature; it also has a significant impact on tubule diameter which becomes five to ten times smaller, reaching values of 300 nm. The structure of fluorinated tubules has been studied by electron microscopy, small angle X-ray and neutron scattering experiments [96].





**Fig 15.** Tubules formed from a mixed-chain fluorocarbon/hydrocarbon glucophospholipid **22** as observed by cryo-transmission electron microscopy (a) (note the internal aqueous core) (from [96]), and by negative staining (b) (from [99]).

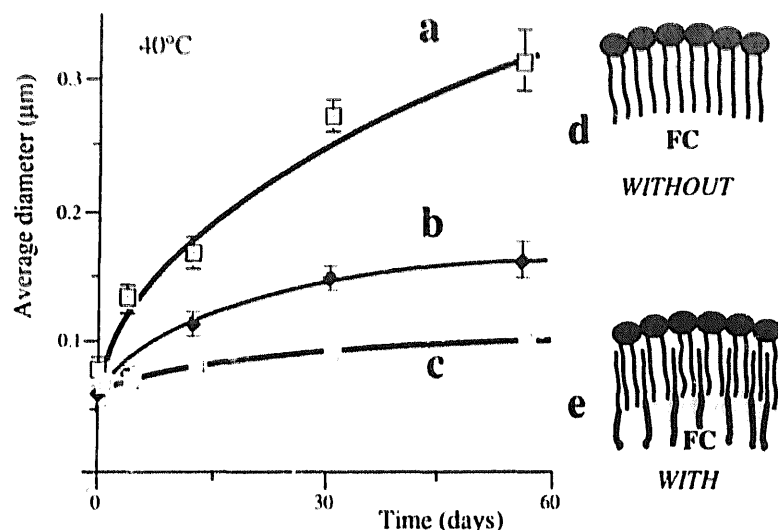
## Colloidal systems with a fluorocarbon phase

### Obtaining stable fluorocarbon emulsions

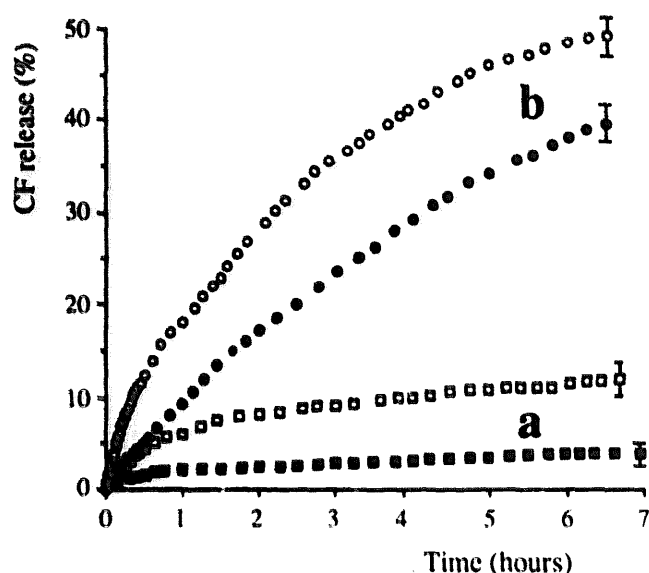
An extensive range of direct, reverse and multiple emulsions, gel-emulsions and microemulsions with a fluorocarbon phase have been reported (fig 6). Achieving stability is a stringent requirement for these emulsions' usefulness and commercial viability. Insufficient stability downed the first fluorocarbon emulsion for oxygen delivery that was approved by the United States Food and Drug Administration in 1989. This emulsion (Fluosol®, Green Cross Corp) had to be stored and shipped frozen and required a cumbersome reconstitution procedure prior to administration [100]. The presently developed, ready-for-use product, Oxygent™ (Alliance Pharmaceutical Corp, San Diego, USA), associates a somewhat lipophilic fluorocarbon, perfluorooctyl

bromide, **1**, with egg yolk phospholipids [4, 48]. Molecular diffusion, the principal cause of particle size increase in sub-micronic fluorocarbon emulsions, is counteracted by adding small amounts of a higher homologue of perfluorooctyl bromide, perfluorodecyl bromide, **2**, which reduces the solubility and diffusibility of the fluorocarbon phase in water [101]. Perfluorotripropylamine, **4**, and perfluoro-*N*-methylcyclohexylpiperidine, **5**, were used for the same purpose in Fluosol and Perftoran, a Russian preparation [102], respectively; these compounds being non-lipophilic have, however, much longer organ retention times. Preserving small particle sizes is also indispensable for achieving effective intravascular persistence and to reduce side-effects related to phagocytosis and macrophage activation [4, 103].

Another highly effective means to stabilize fluorocarbon-in-water emulsions and to control their particle size distribution is to supplement standard phospholipids with



**Fig 16.** Particle size increase at 40°C in a fluorocarbon/phospholipid emulsion (90% w/v perfluorooctyl bromide; 4% w/v egg-yolk phospholipids (EYP)), emulsified with EYP alone (a); stabilized with equimolar amounts of EYP and C<sub>16</sub>F<sub>34</sub> (b); stabilized with equimolar amounts of EYP and C<sub>6</sub>F<sub>13</sub>C<sub>10</sub>H<sub>21</sub> (c). C<sub>16</sub>F<sub>34</sub> and C<sub>6</sub>F<sub>13</sub>C<sub>10</sub>H<sub>21</sub> have close to identical boiling points and should have comparable molecular diffusion-prevention effects according to the Lifshitz-Slezov equation [4]. Hypothetical 'dowel effect' of a mixed fluorocarbon-hydrocarbon diblock compound at the fluorocarbon-phospholipid film interface [36]: without (d), and with C<sub>6</sub>F<sub>13</sub>C<sub>10</sub>H<sub>21</sub> (e). From [36, 106].



**Fig 17.** Release of 5,6-carboxyfluorescein encapsulated in the internal water droplets of water-in-fluorocarbon reverse emulsions (a) and water-in-hydrocarbon reverse emulsions (b). ■, Perfluorooctyl bromide/fluorinated dimorpholinophosphate **13** (F8C11DMP); □, perfluorooctylethane/F8C11DMP; ●, octyl bromide/Span 80; ○, isopropyl myristate/Span 80. Fluorocarbons, which constitute a barrier to the diffusion of the hydrophilic probe, can help control the release of content. From [108].

mixed fluorocarbon-hydrocarbon diblock compounds such as **6** or **7** [36]. The diblock compounds are believed to concentrate primarily at the interface between the fluorocarbon droplets and the film of phospholipids that surrounds them (fig 16), acting as molecular 'dowels' at this interface [104–106]. A clean room equipped with high pressure homogenizers has been built at the Institut Charles Sadron, which

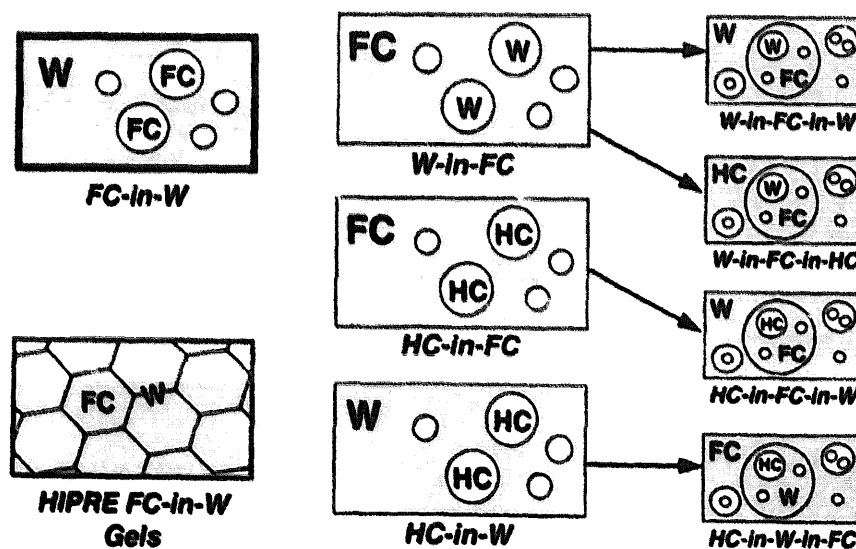
allows the preparation of emulsions of this type, tailor-made for biomedical research.

#### Reverse and multiple emulsions

Reverse, water-in-fluorocarbon emulsions have also been devised. The challenge was to stabilize dispersions of water in one of the most hydrophobic media that exists. In addition, molecular diffusion of water in the fluorocarbon phase, which leads to Ostwald ripening, is facilitated by the extremely low intermolecular cohesion of liquid fluorocarbons. Very stable reverse emulsions were eventually obtained using the highly fluorophilic neutral surfactant **13** ( $n = 8$ ,  $m = 11$ ) [107]. Further stability was gained by adding a salt to the aqueous internal phase. A range of drugs, including antibacterials, bronchodilators, mucolytic, tuberculostatic, cholinergic and antineoplastic agents, etc, were incorporated in these emulsions without loss of stability. Release of 5,6-carboxyfluorescein from such emulsions was shown to be much slower than from water-in-hydrocarbon oil emulsions (fig 17) [108].

Totally apolar hydrocarbon-in-fluorocarbon emulsions, which may be useful for the delivery and controlled release of lipidic materials, have now also been obtained. The amphiphile used to prepare and stabilize such totally apolar emulsions consists again in simple semi-fluorinated alkanes of the F $n$ H $m$  type [37].

These direct and reverse emulsions also led to a range of multiple emulsions, including some novel combinations that involve three distinct non-miscible phases: a fluorocarbon, a hydrocarbon and water (fig 18) [109, 110]. For example, an internal hydrocarbon phase can be separated from the continuous fluorocarbon medium by an intermediate aqueous phase; both lipophilic and hydrophilic drugs may be loaded in such multicompartiment systems.



**Fig 18.** Direct and reverse fluorocarbon emulsions lead to a variety of multiple emulsions, some of which may involve three non-miscible phases: a fluorocarbon, a hydrocarbon, and water.



### Fluorocarbon gels

Gelifying fluorocarbons has been another serious challenge. Fluorocarbons are indeed extremely fluid, mobile liquids with very weak cohesive forces, and they do not dissolve the usual gelifying agents. Nevertheless several types of gels have been reported [111]. Some of these gels are very rich in water and consist of a dispersion of water droplets in an external phase which is actually a water-in-fluorocarbon microemulsion; the surfactants used were perfluoroalkylated ethoxylated alcohols [112, 113].

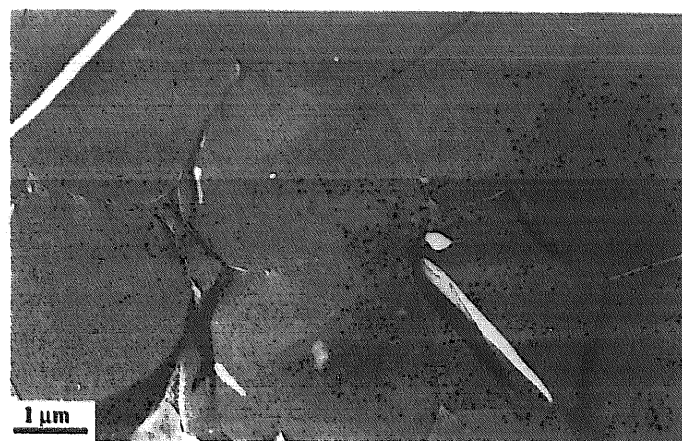
On the other end of the composition spectrum, stable rigid and transparent gels containing up to 99% fluorocarbon could be prepared from a range of fluorocarbons with a wide spectrum of boiling points, using a very low amount of the perfluoroalkylated amine oxide **26** as the surfactant [114]. These gels consist of a high internal phase ratio fluorocarbon-in-water emulsion and have a compartmentalized structure with polyhedral fluorocarbon domains separated by a reverse, hydrated film of surfactant (fig 19). Another type of fluorocarbon-rich gels, in which the fluorocarbon is the continuous phase, were obtained by dispersing a mixture of phospholipids and fluorocarbon-hydrocarbon diblocks in the fluorocarbon, and then adding small amounts of water. Rapid gelification occurs, which is believed to be due to the formation and rapid growth of long, worm-like entangled micelles of surfactants [115].

### Examples of applications in the biomedical field

#### Tools for biomedical research

Few studies on the use of fluorosurfactants for protein extraction have been reported to date. Although fluorinated chains augment surface activity, fluorinated amphiphiles appear to be less detergent toward membranes than their hydrogenated counterparts. This was already indicated by lesser hemolytic activity. Lower protein solubilization potency was observed for compound **17** ( $R_F = C_{10}F_{21}$ ,  $P = 4$ ), in comparison to its hydrocarbon analog or to Triton X-100 [116]. An early claim according to which the phosphocholine derivative **11** ( $n = 8$ ,  $m = 2$ ) could extract a flavoprotein-cytochrome  $b_{558}$  complex without denaturation [117] was not substantiated by further studies [118]. The data available so far thus indicate that fluorinated surfactants are only poorly or not effective for protein extraction.

An astute molecular device, **28**, was built that takes advantage of the tendency of fluorocarbon segments to cluster, in order to create separate zones in which to confine moieties which belong to the same molecule and have distinct functions [119]. Compound **28** is destined to function as a protein recognition device and has an internal *F*-alkylated segment that separates a lipophilic retinoid ligand from a lipidic tail. The fluorocarbon film that forms by aggregation of the *F*-alkyl segments keeps the ligand exposed

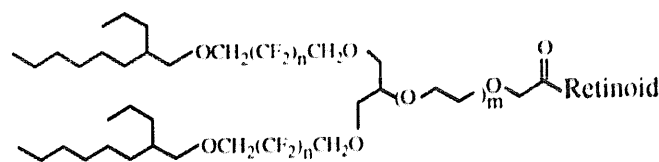


**Fig 19.** Freeze fracture transmission electron micrograph of a high internal phase ratio (98.5%) emulsion of a fluorocarbon (APF-260, Air Products) in water, showing the polyhedral liquid foam domains (micrograph: T Gulik Krzywicki). The surfactant utilized is the fluorinated amine oxide **26**. The emulsion presents itself as a stable, transparent viscoelastic gel. From [114].

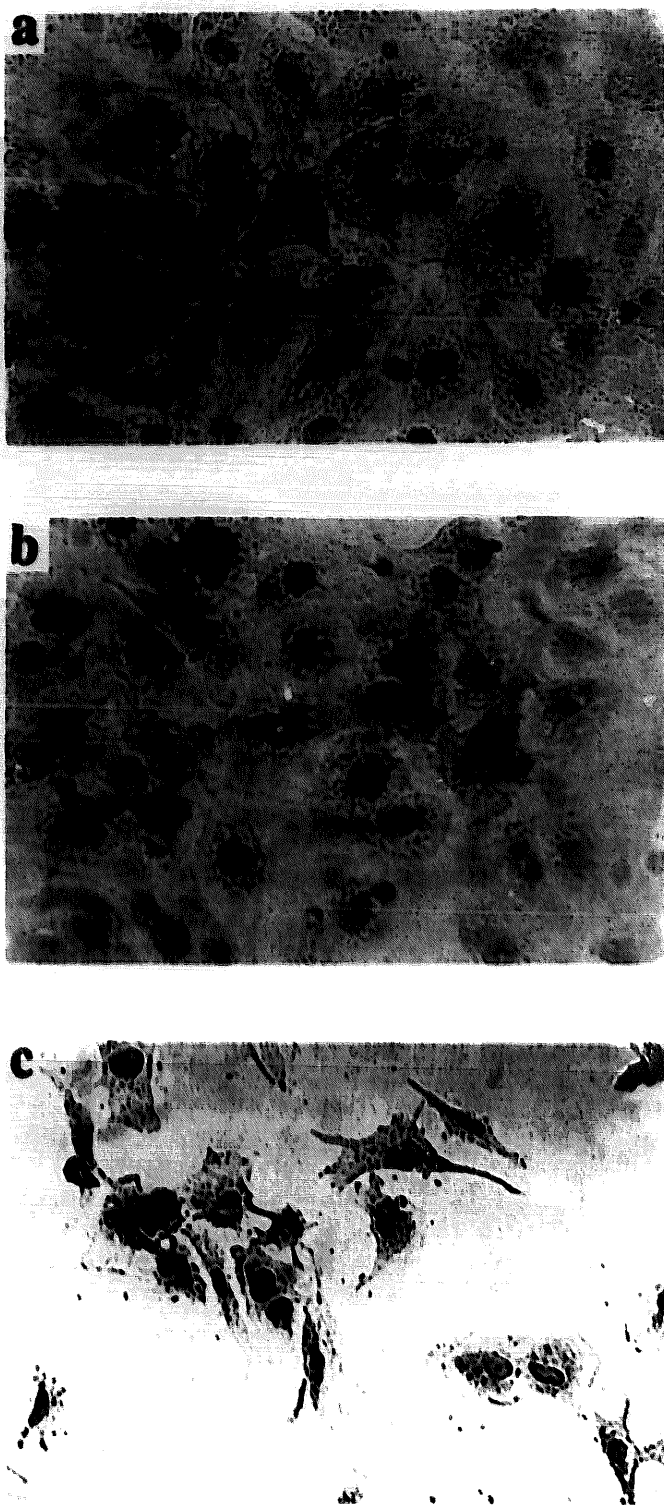
on the aqueous side of a monolayer of the amphiphile and prevents it from being buried in the lipid region of the monolayer. Incubation with a retinoid receptor showed that the macromolecule was efficiently and specifically bound and concentrated onto the lipid film, which was not the case when a non-fluorinated analog of **28** was used (scheme 1).

Gel-like network structures with well-defined mesh size were obtained from fluorocarbon-tailed polyethylene glycols. These gels were used for electrophoretic DNA sequencing in capillary columns [120]. A fluorocarbon affinity emulsion was produced by derivatization with affinity ligands of an *in situ* cross-linked poly(vinylalcohol) emulsifier and used for the extraction of human serum albumin from plasma, or of glucose-6-phosphate dehydrogenase from homogenized baker's yeast [121].

*In vitro* DNA transfection efficacy was not significantly affected when lipospermines with fluorinated lipid tail ends, rather than hydrocarbon ends, were used. This provides evidence indicating that cationic lipid mixing with cellular lipids may not be an important part of the gene transfer process [122]. In another approach a neat fluorocarbon was used for achieving homogenous delivery of a gene-loaded adenoviral vector to the lungs of rabbits [123]. Both gene expression and gene expression distribution throughout the lungs were improved as compared to a saline control.



**Scheme 1.** Structure **28**.



**Fig 20.** Cultured human endothelial cells incubated with a fluorocarbon emulsion stabilized by mixed fluorocarbon-hydrocarbon diblocks (a); no cytotoxicity was observed with regards to controls (b). On the other hand, the usual UW (University of Wisconsin) organ preservation solution was toxic to cells (c). From [126].

Fluorocarbons have been utilized for improving and regulating oxygen supply and pH (through CO<sub>2</sub> delivery) in a variety of cell cultures [124]. Normal cell division and increase in cell density were reported. Mechanical damage caused to cells can be reduced by stirring the culture through the underlying fluorocarbon phase [125]. Protection of alveolar cells against destruction by activated polymorphonuclear granulocytes was noted when the cells were grown on an oxygenated layer of perfluorooctyl bromide [126]. Cell cultures have also been utilized to assess the toxicity of crude fluorocarbons and monitor their purification [127, 128].

Tissue and organ preservation is also currently being investigated. Fluorocarbon emulsions can help prolong the viability of isolated tissue- and organ-based models utilized in physiological studies, including in normothermic conditions. Embryonic chick heart tissue was grown on a fluorocarbon-supplemented medium [129]. An oxygenated perfluorooctyl bromide emulsion was more effective than a suspension of fresh bovine erythrocytes in preserving an *in vitro* preparation of the rat small intestine [130]. Aerobic normothermic preservation of blocks of organs, comprising heart-lung, liver, pancreas, kidney and small intestine was achieved [131]. A fluorocarbon emulsion-perfused pig kidney model allowed investigation of the xenogenic cellular rejection that occurs when the kidneys are perfused with human peripheral blood lymphocytes [132]. The emulsions were of the diblock-stabilized type [36]. The effect of such fluorocarbon emulsions on cultured human endothelial cells was investigated as a preliminary step to achieving warm organ preservation [133]. It was found that the fluorocarbon emulsions were devoid of cytotoxicity. By contrast, the classical organ preservation solutions, UW (University of Wisconsin) and EC (Eurocollins), were toxic to the cells, probably due to their high K<sup>+</sup> concentrations (fig 20).

The diblock-stabilized fluorocarbon emulsions that allowed reproducible, stable preservation of the intestine's neurogenic motor pattern (known as the most sensitive to hypoxia) [130], were recently used to perfuse intestinal preparations of mice in which a gene depletion had been induced (knockout mice) (Bouley L *et al.* in preparation). This model may be useful for the investigation of certain diseases of the central nervous system such as porphyry. It has also potential in pharmaceutical research for the screening of drugs.

#### *Therapeutic oxygen delivery*

One of the major fluorocarbon-based products under clinical development is an injectable fluorocarbon-in-water emulsion for delivering oxygen to patients at risk of tissue hypoxia [43–48]. Recently completed Phase II clinical trials with Oxygen™ demonstrated that the product, when used in conjunction with acute normovolemic hemodilution during surgery, was more effective than blood in reversing transfusion triggers, and delayed the need for a subsequent transfusion significantly longer than blood [134]. Another

fluorocarbon emulsion (Perftoran®, Perftoran Co, Puschino, Russia) has been approved for use in Russia [102]. An emulsion based on  $\alpha,\omega$ -dichloroperfluorooctane **3** (fig 1), Oxyfluor (Hemagen-PFC, St Louis), is in early clinical trials [135].

Using such emulsions as a delivery system for oxygen also shows potential for protecting tissues during transient anemia resulting from trauma; when blood flow is restricted during conditions such as myocardial infarction or stroke; for use during cardiopulmonary bypass surgery [136]; to prevent reperfusion injury; to improve oxygen delivery to certain tumors, rendering these tumors more responsive to radiation and chemotherapy, etc [4, 43–46, 137, 138].

A neat fluorocarbon (Liquivent®, Alliance Pharmaceutical Corp) is currently being clinically evaluated for treatment of respiratory distress through liquid ventilation [49, 50]. The fluid and effectively spreading, O<sub>2</sub>/CO<sub>2</sub>-carrying liquid, reduces a patient's exposure to the harmful effects of conventional mechanical ventilation and helps to recruit collapsed alveoli, improving both lung compliance and oxygenation.

### *Contrast agents for diagnosis*

Fluorocarbons provide the first effective (*ie* stable-enough in the circulation) contrast agent for ultrasound imaging. One such agent, Optison™ (Molecular Biosystems Inc, San Diego, CA), which consists in microbubbles of perfluoropentane within an albumin shell [139], has just been approved for use in the United States (December 1997). Several more products are in advanced clinical trials. MRX-115 (ImaRx Pharmaceutical Corp, Tucson, AZ) is made of lipid encapsulated perfluoropentane [140]. Another agent, Echogen® (Sonus Pharmaceuticals, Bothel, WA), is an emulsion of perfluoropentane whose droplets convert, upon activation, into gas bubbles at body temperature [141]. It is noteworthy that this emulsion is stabilized using a fluorosurfactant, which is, as far as we know, the first intravascular use of a fluorosurfactant in humans. Still another product, Imagent® US (Alliance/Schering AG, Berlin, Germany), takes advantage of the very low solubility of fluorocarbons in water to osmotically stabilize micron-size gas bubbles using perfluorohexane [142]. When injected in the vasculature, these microbubbles function as reflectors for ultrasound, allowing, for example, the assessment of cardiac function, diagnosis of perfusion abnormalities, and detection of tumors in organs [143].

Externally applied fluorocarbon-filled pads (SatPad® Alliance Pharmaceutical Co, San Diego, USA) are now commercially available which improve magnetic resonance (MR) image quality by increasing magnetic homogeneity when fat saturation techniques are utilized [144]. A fluorocarbon has been approved in the United States for oral use as a bowel marker for MR imaging; in this case it is the absence of signal which creates the desired contrast [145].

### *Drug delivery and miscellaneous uses of fluorocarbons and highly fluorinated systems*

A variety of neat fluorocarbons, including perfluorodecalin, perfluorooctyl bromide, perfluoroperhydrophenanthrene and the diblock **8**, are being used in ophthalmology as a tamponade to manage complicated retinal detachments [146–149]. As indicated earlier, fluorinated vesicles and other self-assemblies of fluorosurfactants, and emulsions, gel-emulsions and multiple emulsions with a fluorocarbon phase offer a range of multicompartimentalized vehicles that may be useful for drug delivery [25–31].

Fluorinated surfactants may play a unique role as formulating agents when extreme surface activity, high fluorophilicity, or resistance to an aggressive biological environment is required. Covalently grafting a cleavable fluorocarbon chain onto a drug promotes the self-assembly of the resulting amphiphile or its incorporation into a vesicular membrane, and may help controlling the targeting and delivery of the drug. Bioactive molecules, targeting devices and markers can, for example, be grafted simultaneously onto small amphiphilic telomers [150]. The *F*-alkyl chain enhances the self-aggregation of the prodrug. The amphiphiles' polar head can obviously play a role in targeting. Glycolipidic fluorosurfactants may, for example, be used to achieve targeting through specific recognition by membrane lectins of carbohydrates arranged at the surface of vesicles. The use of fluorosurfactants for stabilizing micronized inhalation drug suspensions for metered drug inhaler has been proposed [151, 152]. Fluorosurfactants also made possible the preparation of microemulsions of water in a supercritical CO<sub>2</sub> continuous phase [153, 154].

### **Perspectives**

The potential of fluorocarbons in the biomedical field appears to be well established. Fluorocarbons are uniquely suited for the formulation and *in vivo* transport of oxygen and carbon dioxide; these transport properties could also be applied to other gases such as nitric oxide. Other products for therapeutic and diagnostic uses, that depend on physical characteristics or combinations of characteristics specific to fluorocarbons, are being developed or investigated. Further applications are likely to emerge that will take advantage of these features.

Water-in-fluorocarbon emulsions, lipid-in-fluorocarbon emulsions and multiple emulsions with a fluorocarbon continuous phase have potential for use in pulmonary drug delivery. This application represents a logical extension of the use of fluorocarbons in liquid ventilation. The reverse emulsions should allow uniform and reproducible distribution of active agents throughout the lung, including the dependent regions, and may provide improved control over drug release. Fluorocarbon gels can provide very thin, water- and fat-repellent, gas-permeable films that may find topical ap-

plications as wound healing ointments, protective barrier creams, as well as use in cosmetics.

The incorporation of a fluorocarbon chain in a molecule can drive the amphiphilic character of this molecule to extremes, resulting in surface activities that cannot be attained with non-fluorinated fatty tails. Fluorosurfactants may therefore find uses wherever surface chemistry is critical. Because of considerably higher effectiveness and efficiency, fluorosurfactants can usually be used in far smaller quantities than standard, non-fluorinated surfactants. This can result in favorable efficacy/toxicity ratios, which may offset the fluorosurfactants' higher cost.

Fluorosurfactants offer the ultimate in terms of hydrophobic effect, which provides potent driving forces for such amphiphiles to self-assemble into supramolecular constructs. The fact that, in addition to being hydrophobic, *F*-alkyl chains are lipophobic is a unique attribute that has been barely exploited. Fluorosurfactants can provide building blocks of supramolecular constructs when extreme segregation between distinct domains (hydrophilic and lipophilic, or both hydrophilic, or both lipophilic) is desired. They provide an additional tool for the chemist for programming the 'self'-assembling of molecules.

Generally speaking, fluorinated amphiphiles constitute novel, versatile components useful for preparing films and membranes; elaborating and stabilizing colloidal systems; coating and dispersing micro- and nano-particles; protecting large bioengineered molecules; modulating the response of temperature and pH-sensitive materials, etc.

Self-association of fluorosurfactants in discrete objects, such as vesicles and tubules, occurs with molecules that are often structurally simpler (shorter, single-tailed, non-chiral, non-hydrogen-bonding) than in the hydrocarbon series. This should facilitate the establishment of structure/property relations, and the elucidation and understanding of the role of individual contributors to a given self-association phenomenon.

The fact that stable organization of fluorinated amphiphiles, especially at interfaces, can be achieved for significantly shorter chain length than for hydrocarbon analogs, should open the access to thinner, yet stable, Langmuir and Langmuir-Blodgett films, and membranes.

Building a fluorinated film within a bilayer membrane has a number of interesting consequences that remain to be exploited. Not only can this film strongly stabilize the membrane, but it can also impact on phase transitions, modify the membrane's curvature, reduce the membrane's permeability to both hydrophilic and lipophilic agents, and influence the behavior of a particle in a biological environment. The fact that little energy is usually needed to induce the formation of fluorinated vesicles may be an advantage when the material to be encapsulated is fragile. The observation that the internal fluorinated film can affect the *in vivo* recognition of particles or the enzymatic attack of the membrane is intriguing and deserves further investigation.

Fluorinated vesicles are usually less permeant to entrapped materials than hydrocarbon vesicles. They may be used for targeting and delivering drugs, prodrugs, contrast agents, immunoactive material, genetic material, etc. They may, as needed, be fitted with markers and targeting devices, or rendered pH-, pressure- or temperature-sensitive.

Fluorosurfactants also allow the preparation of a variety of stable, dispersed, micro- and nano-compartmentalized systems with a fluorocarbon phase, such as emulsions, microemulsions, multiple emulsions, etc. Colloidal systems with three distinct, immiscible phases can be made.

In addition to use as drug delivery devices, such systems could find uses as templates for the elaboration, possibly after polymerization or metallization, of new microporous or microcompartmentalized materials useful as controlled-release systems for other materials, as catalysers, molecular sieves, new composite materials for use in electrooptics and microelectronics. In polymerization chemistry they may provide a means of separating, at the nanometer level, zones that need to be polymerized separately.

The analogy that exists between fluorocarbons and gases, as a consequence of low van der Waals forces, could be further exploited, as for preparing dispersed systems in supercritical fluids and, in particular, in CO<sub>2</sub>. Fluorosurfactants are likely to be indispensable for ensuring the stability of such dispersions.

The simple mixed fluorocarbon-hydrocarbon diblock amphiphiles should find novel uses in colloid chemistry. Fluorocarbon-in-water emulsion stabilization using F<sub>n</sub>H<sub>m</sub> diblocks may provide the basis for a future generation of injectable oxygen carriers. Such emulsions are valuable in biomedical research, for example for stabilizing and extending the viability of experimental models based on isolated tissues and organs. They could allow warm storage of organs destined for transplantation, thus preventing damage resulting from reoxygenation after hypoxia.

The ability of the F<sub>n</sub>H<sub>m</sub> diblocks to combine with standard hydrogenated amphiphiles, particularly phospholipids, and improve their performances, strengthen bilayer membranes, and modulate their properties, is attractive. Further exploration of their membrane ordering and stabilizing capacity should undoubtedly be pursued. The diblocks have the advantage over regular fluorosurfactants of being easy to synthesize and should present a simplified pharmacology.

In contrast to fluorocarbons, the pharmacology of fluorosurfactants is still in its infancy. The available, though limited, preliminary acute toxicity data are encouraging. The spectacular reduction or suppression of hemolysis when fluorinated chains are introduced in surfactants, is remarkable. Absorption, distribution, metabolism and excretion studies are badly needed. The diversity of recently synthesized, well-defined fluorosurfactants should facilitate this task. The results of these studies will largely determine the extent to which fluorosurfactants may be used in pharmaceuticals; the acceptable level of side effects will of

course also depend on the indication, dose, and route and regimen of administration.

The limited information available on membrane protein extraction using fluorosurfactants indicates lesser effectiveness than for hydrocarbon surfactants or no effectiveness at all, probably due to the lipophobic character of the perfluoroalkyl tails.

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